ABIOTIC STRESS EFFECTS ON PHYSIOLOGICAL, AGRONOMIC AND MOLECULAR PARAMETERS OF 1-MCP TREATED COTTON PLANTS

A Dissertation

by

VLADIMIR AZEVEDO DA COSTA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2010

Major Subject: Agronomy

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Approved by:

Chair of Committee,	J. Tom Cothren
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ABSTRACT

Abiotic Stress Effects on Physiological, Agronomic and Molecular Parameters of 1-MCP Treated Cotton Plants. (December 2010)

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Chair of Advisory Committee: Dr. J. Tom Cothren

Abiotic stresses impact cotton (*Gossypium hirsutum* L.) affecting physiological, molecular, morphological, and agronomic parameters. One of the main yield components in cotton production is the number of bolls per unit area. However, boll abortion is increased when cotton experiences various stresses during its reproductive development that can consequently reduce lint yield. Prior to abscission, a burst in ethylene is observed which may be assumed to be the signal necessary to initiate abscission of that particular structure. It is desirable to prevent fruit loss that may be induced by the peak in ethylene prior to abscission. One potential option to cope with the loss of cotton reproductive structures is the use of ethylene inhibitors. The overall objective of this research was to establish if 1-MCP would synergize, ameliorate, or overcome the effects of abiotic stresses on physiological, molecular, morphological, and agronomic parameters of cotton plants under abiotic stress conditions in field and greenhouse studies. Field and greenhouse experiments were conducted from 2007 to 2009 as a randomized complete block design with four replications in the field, and as a

2x2 factorial design in a split-block arrangement with five replications in the greenhouse. Field treatments consisted of three rates of 1-MCP (0, 25 and 50 g a.i. ha^{-1}) in combination with a surfactant applied at mid-bloom. One day later, ethephon (synthetic ethylene) was applied as a source of abiotic stress. Greenhouse treatments were two 1-MCP rates (0 and 2.4 g a.i. L^{-1}) during a14-h overnight incubation that were then subjected to two water regimes (control and stressed) as the source of stress. Greenhouse assessments with gas exchange analysis revealed that water deficit stress started to impact plants at a moderate water stress, 5 days after 1-MCP treatment (DAT) and a water potential (ψ_w) of -1.4 MPa. The 1-MCP increased water use efficiency in well-watered plants at 1 DAT. Many of the yield components, plant mapping, and biomass parameters investigated were detrimentally affected by drought. However, drought increased specific leaf weight, chlorophyll content, and harvest index. The 1-MCP improved reproductive node numbers mainly during drought, but did not lead to a better harvest index, since 1-MCP caused high abscission. Ethylene synthesis and molecular investigations in greenhouse conditions showed that at 1, 5, 7, 9, 11, and 13 DAT, ethylene production of stressed plants never exceeded those of control plants. As the ψ_w became more negative ethylene production rate was reduced among stressed plants independent of 1-MCP treatments. However, at 1 DAT 1-MCP caused a transient climacteric stage (ethylene synthesis increase) in leaves. The two primary genes associated with ethylene synthesis, *ACS6* (1-aminocyclopropane-1-carboxylic acid synthase) and *ACO2* (1-aminocyclopropane-1-carboxylic acid oxidase) expression generally showed an identical trend that supported the ethylene synthesis data. The 1-

MCP did not ameliorate any of the detrimental effects of water stress on gas exchange at the point where it started to impact cotton plants. 1-MCP had little or no positive effect on plant mapping, dry matter partitioning and chlorophyll content. Field investigations revealed that at harvest, fruit set in the upper portion of the canopy was influenced by 1-MCP. This portion of the canopy had a greater number of full size, yet immature bolls, which potentially could have had a positive influence on the lint yield. However, ethephon caused the highest lint yield since ethephon treated plants had more open bolls and total bolls in the lower canopy at harvest.

DEDICATION

I dedicate this dissertation to God for always being my light and strength. To my mom, Maringá, for her love, incentive, determination and constant support of my decisions teaching me that anything worthwhile in life takes time, and dedication. To my wife, Anita, for all of her love, patience, encouragement, inspiration, and support she has given, providing the meaning for my hard work. I also dedicate this dissertation to my beautiful and good daughter, Isabella, whose smile and love brightens each day. I would like to extend this dedication to my grandparents, Lidia and Clódio, my brother, Cristiano, my father, Frederico, and all my Polish family. Thank you all for your unending support, willingness to accept, and eagerness to love me.

Eu dedico esta dissertação a Deus por ser minha luz e força. À minha mainha, Maringá, por seu amor, incentivo, determinação e suporte constante às minhas decisões ensinando-me que tudo que vale a pena na vida leva tempo, e necessita de plena dedicação. À minha esposa, Anita, por todo seu amor, paciência, estímulo, inspiração, e suporte, provendo o significado do meu trabalho árduo. Eu também dedico esta dissertação à minha filha, Isabella, cujo sorriso e amor iluminam cada dia. Eu gostaria de extender esta dedicação aos meus avós, Lidia e Clódio, meu irmão, Cristiano, meu pai, Frederico, e toda a minha família Polonesa. Obrigado a todos pelo suporte interminável, disposição a aceitar, e avidez em amar-me.

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CHAPTER I

INTRODUCTION

In recent years drought stress tolerance has become one of the main points of interest to agronomic research since major crops as cotton are experiencing drier years than normal due to changes in weather patterns (Gowda et al., 2007; Pettigrew, 2004a) and declining irrigation reserves which occur together with an increase in costs associated with irrigation (Gowda et al., 2007) as water supplies from aquifers are dwindling in part due to limited recharge (Howell et al., 2004).

Water deficit stress causes detrimental impact in cotton production (Howell et al., 2004; Mooney et al., 1991; Pettigrew, 2004b). Studies show that even though cotton is able to maintain a leaf turgor potential (ψ_t) by osmotic adjustments while facing moisture deficit, it eventually faces a reduction in leaf water potential (ψ_{wl}) under dry conditions (Nepomuceno et al., 1998; Turner et al., 1986). In response to drought, stomata tend to close reducing their conductance, consequently affecting leaf photosynthesis (Ephrath et al., 1990; Faver et al., 1996; Genty et al., 1987). When under water stress, overall dry matter accumulation in cotton plants is decreased (Mooney et al., 1991) and expansion of leaf blades and plant growth is reduced, promoting stunted growth (Ball et al., 1994; Gerik et al., 1996). Limited water availability causes cotton plants to generate fewer flowers resulting in reduced boll production. Moreover, stress is

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severe during reproductive development boll abortion is increased, reducing lint yield (Gerik et al., 1996; Pettigrew, 2004a; Turner et al., 1986). It has been established that the variable which contributed the most to lint yield was the number of bolls area^{-1} (Boquet et al., 2004; Worley et al., 1974; Wu et al., 2005). However, boll abortion is increased when cotton plants are under severe stress during their reproductive development that consequently, reduces lint yield (Gerik et al., 1996; Pettigrew, 2004a; Turner et al., 1986). Morgan et al. (1992) observed that there was a burst in ethylene sysnthesis that lasted four days prior to occurrence of abscission. The authors suggested that this peak in ethylene may be the signal necessary to initiate cell wall hydrolysis in the abscission zone followed by abscission of that particular structure.

The literature presents diverging opinions on the impact of water deficit on ethylene synthesis. Experiments reporting increased ethylene synthesis due to water stress used detached plant parts that were subjected to a rapid dry down period and then stored in closed chambers while air samples were collected for ethylene measurements (Adato and Gazit, 1974; Aharoni, 1978; Apelbaum and Yang, 1981; Ben-Yehoshua and Aloni, 1974; Bergner and Teichmann, 1993; Hoffman et al., 1983; Huberman et al., 1993; McKeon et al., 1982; McMichael et al., 1972; Michelozzi et al., 1995; Narayana et al., 1991; Tudela and Primo-Millo, 1992; Wright, 1981; Wright, 1977). On the other hand, ethylene emission studies which exposed plants to a gradual dry down period by terminating watering and collecting air samples from intact plants or plant parts placed in closed chambers with or without constant air flow indicated that water deficit stress did not increase ethylene production (Ben-Yehoshua and Aloni, 1974; Eklund et al.,

1992; Feng and Barker, 1992; Hubick et al., 1986; Morgan et al., 1990; Narayana et al., 1991).

Two key enzymes are involved in the ethylene synthesis pathway. The first enzyme ACC-synthase (ACS) converts *S*-adenosylmethionine (SAM), which originates in the methionine cycle, to 1-aminocyclopropane-1-carboxylic acid (ACC). ACC is then oxidized to ethylene by ACC-oxidase, ACO (Chaves and Mello-Farias, 2006; Kende, 1993; Zarembinski and Theologis, 1994). Tissues that do not produce significant levels of ethylene have low ACS activity, but upon stimulation ACS activity can be quickly induced (Chae et al., 2003). Both ACS and ACO can be induced upon stress (Morgan and Drew, 1997). Unlike ACS, ACO has a constitutive activity present in most tissues. Thus, one of the major steps during ethylene induction is ACS, which is a rate-limiting enzyme (Chae et al., 2003). The *ACS6* gene encodes for one of the ACS proteins and is part of a multi-gene family (Fluhr and Mattoo, 1996; Kende, 1993; Tsuchisaka and Theologis, 2004) in which all genes are independently regulated (Fluhr and Mattoo, 1996). *ACO2* also belongs to a multi-gene family encoding ACO proteins (Barry et al., 1996; Kende, 1993). Ethylene perception occurs when the plant hormone binds to an ethylene receptor (ETR). ETRs are a family of membrane receptors (Chang et al., 1993), and *ETR5* gene encodes for a membrane protein which is part of this multi-gene family. Ethylene perception and its signal transduction pathway that follows are feedback regulated (Urao et al., 2000).

It is desirable to protect yield by preventing fruit loss induced by the peak in ethylene prior to abscission. It is necessary to look for alternatives that could reduce or

prevent abortion of cotton bolls under stress. Preventing loss of flowers and young fruit is essential in cotton yield enhancement (Heitholt et al., 1993); thus, ethylene inhibitors could provide an alternative for coping with the loss of reproductive structures, in an effort to improve cotton yield.

The compound 1-methylcyclopropene (1-MCP) is a gaseous ethylene antagonist that blocks ethylene receptors, consequently inhibiting its perception and preventing ethylene effects in the plant tissues (Blankenship and Dole, 2003; Sisler and Serek, 1997). The affinity of 1-MCP to ethylene receptors is 10x greater than the affinity of ethylene to its receptors (Blankenship and Dole, 2003). 1-MCP is widely used in horticultural production (Fan and Mattheis, 2000). Studies in horticulture mainly focused on post-harvest physiology of climacteric fruit to counter the detrimental effects of ethylene. These studies showed that the compound impacts a variety of physiological processes, such as decreasing ethylene synthesis (Blankenship and Dole, 2003; Dong et al., 2001; Jeong et al., 2002), respiration (Blankenship and Dole, 2003; Dong et al., 2001; Fan and Mattheis, 2000), and chlorophyll degradation (Blankenship and Dole, 2003; Fan and Mattheis, 2000; Jiang et al., 2002), thus extending shelf-life (Fan and Mattheis, 2000).

With the existing information, studies were established to investigate the following objectives:

1. To establish whether drought affects ethylene biosynthesis and the expression of related involved genes of detached leaves from cotton plants exposed to water deficit stress during the peak reproductive phase.

Secondary objectives were: i) determine if ethylene production, relative gene expression, and leaf expansion could be altered by the presence of 1-MCP treatment in response to drought; ii) confirm if 1-MCP causes a transient increase in ethylene synthesis.

2. To establish how drought affects gas exchange, plant growth/development and yield components of 1-MCP treated cotton plants during the peak of reproductive phase under greenhouse conditions. A secondary objective was to determine if gas exchange, plant growth/development and yield components responses to drought could be altered by the presence of 1-MCP treatment.
3. To determine the impact of 1-MCP on growth and yield components of cotton plants treated with ethephon as a source of abiotic stress under field conditions. A secondary objective was to assess to what extent plants can compensate for fruit loss during the late season.

CHAPTER II

ETHYLENE SYNTHESIS, RELATIVE GENE EXPRESSION, AND LEAF GROWTH IN COTTON UNDER DROUGHT STRESS

OVERVIEW

Literature presents diverging opinions on how water deficit affects ethylene synthesis. Experiments reporting that ethylene synthesis increased due to water deficit stress used detached plant parts subjected to quick drying. To the contrary, ethylene emission studies which evaluated whole plants exposed to a gradual drying period failed to show increases in ethylene levels. The objectives of this study were to determine if the rate of ethylene synthesis and related gene expression is modified by water deficit stress in plants and if 1-methylcyclopropene (1-MCP; an ethylene antagonist) affects ethylene production and gene expression in cotton (*Gossypium hirsutum* L.). Greenhouse studies were conducted during two years as a 2x2 factorial design in a split-block arrangement with five replications. Treatments included two 1-MCP rates (0 and 2.4 μg a.i. L^{-1}) applied during a 14-h overnight that were then subjected to two water regimes (control and stressed). At 1, 5, 7, 9, 11, and 13 days after 1-MCP treatment (DAT), ethylene production of stressed plants never exceeded those of control plants. As the water potential (ψ_w) became more negative the ethylene production rate was reduced in stressed plants independent of 1-MCP treatments. A linear relationship between ψ_w and ethylene was evident after 7 DAT. However, at 1 DAT, 1-MCP caused a transient climacteric stage (ethylene synthesis increase) in cotton leaves, while blocking the ethylene auto-inhibition phase. *ACS6* and *ACO2* expression, which respectively encode

for enzymes that convert *S*-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) and ACC to ethylene, generally showed an identical trend that supported the ethylene synthesis data. GDSL-motif lipase gene, which encodes for multifunctional properties enzymes, showed a potential as a drought-responsive gene. Results indicated that water deficit stress caused a decrease in ethylene synthesis, which was validated in part by gene expression.

INTRODUCTION

Water deficit stress adversely affects cotton production (Howell et al., 2004; Mooney et al., 1991; Pettigrew, 2004b). Water stress responses involve a series of alterations in physiology, metabolism, and morphology that extend from the cellular to the whole plant level (Cellier et al., 1998). At the cellular level, dehydration results in the loss of free-water that increases solute concentration as molecules become more dense; cell turgor is reduced altering cell volume and shape; protein synthesis is inhibited and denaturation progresses; and stability of the plasmalemma, tonoplast and other organelle membranes is lost (Bray, 1997; Hsiao, 1973). Drought responses include reduced stomatal conductance and gas exchange; decreased transpiration; increased leaf temperature; changes in respiration; compromised cell wall synthesis and cell expansion as well as cell division; and alterations in enzyme and hormone levels (Hsiao, 1973). Water stress is detrimental to the overall transport within the plant and subsequent growth. Ion uptake by roots and their transport, as well as translocation of photoassimilates, is reduced; resistance to water flow in the xylem is increased due to cavitation (Hsiao, 1973); and expansion of leaf blades and plant growth is reduced,

promoting stunted growth (Ball et al., 1994; Boyer, 1970; Gerik et al., 1996). Due to the enormity of literature covering water stress responses relative to physiological performance, the focus of this paper is on whether water deficit stress impacts ethylene synthesis and its related genes in cotton.

The literature presents diverging opinions on the impact of water deficit on ethylene synthesis. Experiments reporting increased ethylene synthesis due to water stress used detached plant parts that were subjected to a rapid dry down period and then stored in closed chambers while air samples were collected for ethylene measurements.(Adato and Gazit, 1974; Aharoni, 1978; Apelbaum and Yang, 1981; Ben-Yehoshua and Aloni, 1974; Bergner and Teichmann, 1993; Hoffman et al., 1983; Huberman et al., 1993; McKeon et al., 1982; McMichael et al., 1972; Michelozzi et al., 1995; Narayana et al., 1991; Tudela and Primo-Millo, 1992; Wright, 1981; Wright, 1977) On the other hand, ethylene emission studies which exposed plants to a gradual dry down period by terminating watering and collecting air samples from intact plants or plant parts placed in closed chambers with or without constant air flow indicated that water deficit stress did not increase ethylene production (Ben-Yehoshua and Aloni, 1974; Eklund et al., 1992; Feng and Barker, 1992; Hubick et al., 1986; Morgan et al., 1990; Narayana et al., 1991).

Two key enzymes are involved in the ethylene synthesis pathway. The first enzyme ACC-synthase (ACS) converts *S*-adenosylmethionine (SAM), which originates in the methionine cycle, to 1-aminocyclopropane-1-carboxylic acid (ACC). ACC is then oxidized to ethylene by ACC-oxidase, ACO (Chaves and Mello-Farias, 2006; Kende,

1993; Zarembinski and Theologis, 1994). Tissues that do not produce significant levels of ethylene have low ACS activity, but upon stimulation its activity can be quickly induced (Chae et al., 2003). Both ACS and ACO can be induced upon stress (Morgan and Drew, 1997). Unlike ACS, ACO has a constitutive activity present in most tissues. Thus, one of the major steps during ethylene induction is ACS, which is a rate-limiting enzyme (Chae et al., 2003). The *ACS6* gene encodes for one of the ACS proteins and is part of a multi-gene family (Fluhr and Mattoo, 1996; Kende, 1993; Tsuchisaka and Theologis, 2004) in which all genes are independently regulated(Fluhr and Mattoo, 1996). *ACO2* also belongs to a multi-gene family encoding ACO proteins (Barry et al., 1996; Kende, 1993). Ethylene perception occurs when the plant hormone binds to an ethylene receptor (ETR). ETRs are a family of membrane receptors (Chang et al., 1993), and *ETR5* gene encodes for a membrane protein which is part of this multi-gene family. Ethylene perception and its signal transduction pathway that follows are feedback regulated (Urao et al., 2000).

The compound 1-methylcyclopropene (1-MCP) is a gaseous inhibitor of ethylene action that blocks ethylene receptors (its perception) and prevents ethylene effects in the plant tissues (Blankenship and Dole, 2003; Sisler and Serek, 1997). It is believed that its affinity to ethylene receptors is 10 times greater than the affinity of ethylene to its receptors (Blankenship and Dole, 2003). 1-MCP is widely used in horticulture (Fan and Mattheis, 2000). Studies in horticulture have focused mainly on post-harvest physiology of climacteric fruit for its ability to counter the detrimental effects of ethylene; results of these studies showed that the compound impacts a variety of physiological processes,

such as decreasing respiration (Blankenship and Dole, 2003; Dong et al., 2001; Fan and Mattheis, 2000), chlorophyll degradation (Blankenship and Dole, 2003; Fan and Mattheis, 2000; Jiang et al., 2002), and ethylene synthesis (Blankenship and Dole, 2003; Dong et al., 2001; Jeong et al., 2002), thus extending shelf-life (Fan and Mattheis, 2000). Nevertheless, de Wild et al. (2003) observed short-lived increases in ethylene synthesis following 1-MCP applications in freshly harvested pears while investigating CO_2 effects on ethylene synthesis. They concluded that this increase was due to the direct action of 1-MCP on the autoinhibition phase (preclimacteric period) of ethylene production by ethylene itself.

The primary objective of this study was to establish whether drought affects ethylene biosynthesis and the expression of related involved genes of detached leaves from cotton plants exposed to water deficit stress during their peak reproductive phase. Secondary objectives were: i) to determine if ethylene production, relative gene expression, and leaf expansion could be altered by the presence of 1-MCP treatment in response to drought; and ii) confirm if 1-MCP causes a transient increase in ethylene synthesis.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Cotton (*Gossypium hirsutum* L. cv. 'Americot NexGen 2448 R') seeds were sown in 15.2- L plastic pots filled with 11.5 L of fritted clay. Fritted clay which is sold as Absorb-N-Dry (Balcones Mineral Corp., Flatonia, TX) was chosen as a medium for growing plants for its quick drainage and low dry-bulk density, but mainly for its

capacity to hold large quantities of plant-available water (van Bavel et al., 1978), since it was intended to impose a gradual dry down as a source of moisture deficit. After emergence, seedlings were thinned to two plants per pot, with each located on opposite sides of the pot. All plants were watered thoroughly daily with reverse osmosis (RO) water with an electro-conductivity of 6.7 μS, and fertilized every other week with a complete, water soluble fertilizer containing macro- and micronutrients. Plants were grown in the Borlaug Center greenhouses, at Texas A&M University. Day and night temperatures were 32 and 31 °C, respectively, in 2008 and 27 and 26 °C, respectively in 2009. Day and night relative humidity readings were 57 and 55%, respectively, in 2008 and 41 and 39%, respectively in 2009. These readings were measured with a Center 315 Temperature and Humidity Meter (Center Technology Corp., Taiwan). Midday photosynthetic photon flux density (PPFD) was measured with the quantum sensor of a Li-Cor 6400 XT infrared gas analyzer (LI-COR Inc., Lincoln, NE) and averaged 900 μmol m^{-2} s^{-1} in 2008 and 850 μmol m^{-2} s^{-1} in 2009. The photoperiod was dictated by the natural conditions of the locality (College Station, TX: 30°30'26.74" N, 90°20'58.83" W).

Treatment Application and Experimental Design

The experiment consisted of four treatments with five replications, and was repeated twice. Treatments were initiated when plants reached an average of sixteen mainstem nodes, which approximated the mid-bloom. The potting medium of all plants was brought to container capacity. One hundred and forty pots were randomly chosen and moved to the potting room of the greenhouse facility and placed into 4-m^3 sealed,

polyethylene tents equipped with SmartFresh^{SM} Research Tablet Generators (AgroFresh Inc., PA) and Coleman tent fans (The Coleman Company, Inc., KS) to generate and uniformly deliver the gaseous treatment. This same system is widely used to treat fruits and vegetables commercially. Each one of the two gaseous treatments consisted of 70 pots, no 1-MCP and 2.4 μg a.i. L^{-1}, released from tablets placed into flasks of the SmartFresh^{SM} Research Tablet Generators. Gaseous application of 1-MCP has shown that treatment durations of less than 12 h do not provide sufficient protection (Jeong et al., 2002). Thus, pots were placed inside tents during a 14-h overnight interval, then transported back to the greenhouse tables, and arranged as a split-block in a 2x2 factorial experiment. The same pots were subjected to two watering treatments, as the split-block: 70 pots were subjected to irrigation at container capacity, and water was withheld from the other 70 pots during the 14 days trial. The magnitude of the drought treatments was determined by a preliminary experiment in which pots were watered, and then weighed 1 h later to determine the container capacity. A pot was randomly selected and the two plants were weighed. This weight was then subtracted from the container capacity to determine the container capacity weight of the medium (MCW). At wilting, weight of the medium averaged 8% of the MCW.

Volumetric water content of the upper 6 cm of the potting medium was determined daily prior to beginning measurements by using a HH2 Theta Probe (Delta-T Devices, Cambridge, U.K.) set on the mineral soil setting throughout the experiment. Fifteen randomly selected extra pots were assigned to drought stress and were used to monitor the amount of water lost by evapotranspiration. Each day these pots were

weighed, the weight of two plants (roots plus shoots) was subtracted, and the weight of the medium was determined. When the average weight of the medium had decreased below 8% of the MCW, the amount of water necessary to bring pots slightly above 8% MCW was supplied to these 15 extra pots, to the pots from which plants were collect, and also to all the other 70 pots under water stress treatment. This evapotranspiration check procedure was modified from Starman and Lombardini (2006).

Sampling Procedure

Pots not subjected to the water deficit treatment were watered daily at least 1 h prior to any sampling and data collection. Measurements were initiated on day 1, which coincided with the same day pots were brought back to the greenhouse and subjected to water regime treatments, and one day after 1-MCP treatments were initiated. Measurements/samplings were taken every other day during a 14-d interval between 1100 and 1400 h on 20 pots, with 5 pots replicates being used for each treatment. Plants were destructively sampled once only and then discarded. A new set of 20 pots was used on the next sampling day. Two plants per pot were utilized because it was necessary to remove plant material for two procedures: the uppermost unfurled leaf from one of the plants was utilized for ethylene emission measurements, and the leaf apex from the other plant was used for the ethylene responsive cotton genes analysis. Thus, one destructive sampling did not influence the other since samples were collected from separate plants.

Plant Water Potential

Leaf water potential was determined as outlined by Scholander et al. (1965) using a pressure chamber, in which the third uppermost fully-expanded leaf from one of

the plants per each pot was utilized. Leaves were placed into the chamber within 15 s of excision and the chamber was pressurized at a rate of 0.02 MPa s^{-1} (Turner, 1988).

Leaf Growth

Leaf expansion was investigated in the current research to document the detrimental effect of water stress treatment on cotton plants, since it has been well documented (Masle and Passiowa, 1987; Matsuda and Riazi, 1981) that leaf expansion is very sensitive to water deficit stress and one of the earliest responses to such a stress. Leaf expansion was calculated based on leaf area changes measured every other day starting at 4 d after 1-MCP treatment (DAT). The mainstem uppermost unfurled leaf from one of the plants per each pot was used for this measurement. The leaf growth rate was calculated based on differences in leaf area (Boyer, 1970) before and after a growth period of 48 h until 14 DAT.

Ethylene Emissions

Based on the fact that ethylene emission in cotton follows a circadian pattern that peaks at midday (Jasoni et al., 2002), all samples were taken between 1200 and 1400 h. The blade of the uppermost unfurled leaf from one of the plants per each pot was excised, immediately placed in a 20-mL syringe, and incubated individually for 1 h in 10 mL of headspace. After 1-h incubation, a 1-mL gas aliquot was removed with an air-tight syringe and injected into a gas chromatograph (Beltrano et al., 1997; Morgan et al., 1990; Sisler and Serek, 1997). The Photovac 10S Plus Digital Gas Chromatograph (PHOTOVAC, Inc., MA) used was equipped with a photoionization detector and a Carbopak B HT column, and was calibrated each sampling day with an ethylene

standard prior to sample injection. Leaves used for ethylene emission determinations, were dried for 96 h at a minimum of 72 °C (Goldman et al., 1989; Sánchez-Blanco et al., 2009; Starman and Lombardini, 2006) and dry weights were determined gravimetrically.

Ethylene Responsive Cotton Genes

The genes that were examined included representative genes involved in ethylene production (*ACO2, ACS6*) and perception (*ETR5*) as well as *GDSL.* The relationship of *GDSL* to ethylene is still unknown but has shown to be very responsive to this gas (S. Finlayson, personal communication). The leaf apex from one of the plants from each pot was excised and immediately placed in a coin envelope previously identified by treatment, then immerged in liquid nitrogen, and subsequently stored in a -80 °C freezer for subsequent isolation of RNA. Each leaf apex constituted one replication out of five per treatment. The gene expression procedures were modified from Finlayson et al. (2010). Total cellular mRNA was isolated from 0.09 to 0.11 g of tissue using a Spectrum™ Plant Total RNA Kit (Sigma, St. Louis, MO); concentration per each sample was equalized, then RNA quality was evaluated on glyoxal agarose gels (1%). Subsequently, 6-µg RNA samples were treated with RNase-free DNase (Promega Co., Madison, WI), and reextracted with TRIzol (Invitrogen, San Diego, CA). RNA was transcribed to cDNA using a SuperScript III Kit (Invitrogen, San Diego, CA) and random hexamers. Real-time PCR (10-µL reaction volumes) were run in triplicates with a corresponding – RT (minus-reverse transcriptase) control using SYBR Green PCR Master Mix and the ABI Prism 7900 Sequence Detection System (Applied Biosystems, Foster City, CA). Cloned genes in plasmid vectors were used to generate known

concentration dilution series that were quantified by the same PCR process in order to plot standard curves for each gene (*18 s, ACS6, ACO2, ETR5, and GDSL*). Threshold cycle (CT) values per each reaction generated by the ABI Prism7900 SDS were converted to absolute number of transcripts, which were then normalized by *18 s* transcripts.

Data Analysis

Data were subjected to analysis of variance using PROC MIXED (Littell et al., 2006) of SAS version 9.2 (SAS Institute Inc., 2008) for a split-block design, where main plot was 1-MCP application, and subplot was water regime. Homogeneity of variance across years was tested for each variable. Paired *t*-tests were used to assess differences between two means. Multiple mean comparisons were made using Tukey's test at $P \leq 0.05$ (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Leaf Growth

Leaf expansion was severely inhibited by water deficit stress. By the second day of evaluation (6 DAT), stressed plants (stress, and stress plus 1-MCP) had significantly lower leaf growth than both well-watered plants (control, and control plus 1-MCP). The leaf expansion of stressed plants continued very discreetly until 14 DAT (Fig. II.1). On the other hand, well-watered plants with and without of 1-MCP showed a steep expansion curve from the beginning of measurements up to 10 DAT; after that time, leaf growth ceased since leaves reached their maximum blade expansion (Fig. II.1). At 10 DAT, the leaf sizes of the control and control plus 1-MCP treatments were 162 and 142

cm^2, while stress and stress plus 1-MCP were 54 and 61 cm^2, respectively (Fig. II.1). The 1-MCP seemed to cause an inverse effect on leaf expansion depending on water regime. Under well-watered conditions, it numerically inhibited leaf growth, being significantly inhibited at 10 DAT. Under water-stressed conditions, however, 1-MCP showed a numerical promotion of leaf growth (Fig. II.1).

At 10 DAT, leaf growth rates of well-watered treatments reached their peak: 50 $cm^2\ d^{-1}$ for the control and 41 $cm^2\ d^{-1}$ for the control plus 1-MCP treatments. The values under water stress were 9 $cm^2\ d^{-1}$ without 1-MCP and 12 $cm^2\ d^{-1}$ with 1-MCP (Fig. II.2). After 10 DAT, well-watered plants from both treatments reduced their rate of growth until the end of the evaluations indicating cessation of growth due to the fact that the maximum leaf size was potentially reached (Fig. II.2). At 8 DAT, 1-MCP significantly impacted leaf growth rate depending on the water regime, decreasing the rate while under well-watered conditions and increasing it while water-stressed (Fig. II.2). The water regime also played a significant role in leaf growth rate, which was highly impacted while under water stress, and was declined from the beginning to the end of evaluations (Fig. II.2). Previous research in maize (*Zea mays* L.) (Boyer, 1970; Saab and Sharp, 1989), soybean [*Glycine max* (L.) Merr.] (Boyer, 1970; Meyer and Boyer, 1981), sunflower (*Helianthus annuus* L.) (Boyer, 1970), wheat (*Triticum aestivum* L.) (Passioura, 1988), and cotton (Ball et al., 1994; Gerik et al., 1996; Pettigrew, 2004b; Turner et al., 1986) also reported cessation or decline in leaf growth due to detrimental effects of drought.

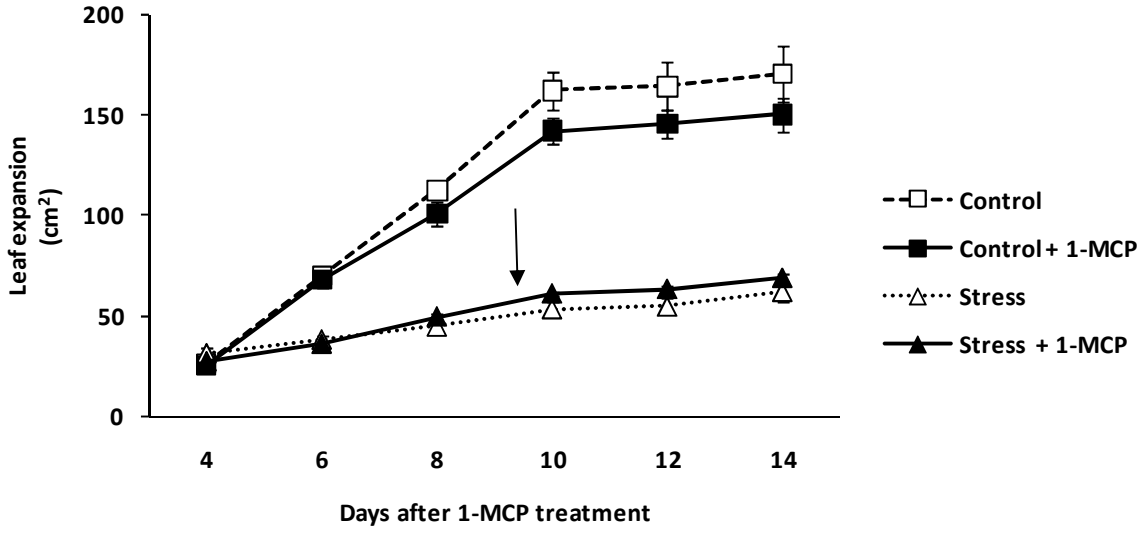

Fig. II.1. Cumulative leaf expansion of well-watered (control, and control plus 1-MCP) and water-stressed (stress, and stress plus 1-MCP) cotton plants in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009. The arrow denotes when watering of stressed plants was initiated with the amount of water necessary to bring pots slightly above 8% MCW. This occurred on the evening of 9 DAT and was resupplied again whenever plants reached water status below the wilting point. Plants were thus kept at a constant level of stress. Bars represent SE where greater than the symbol.

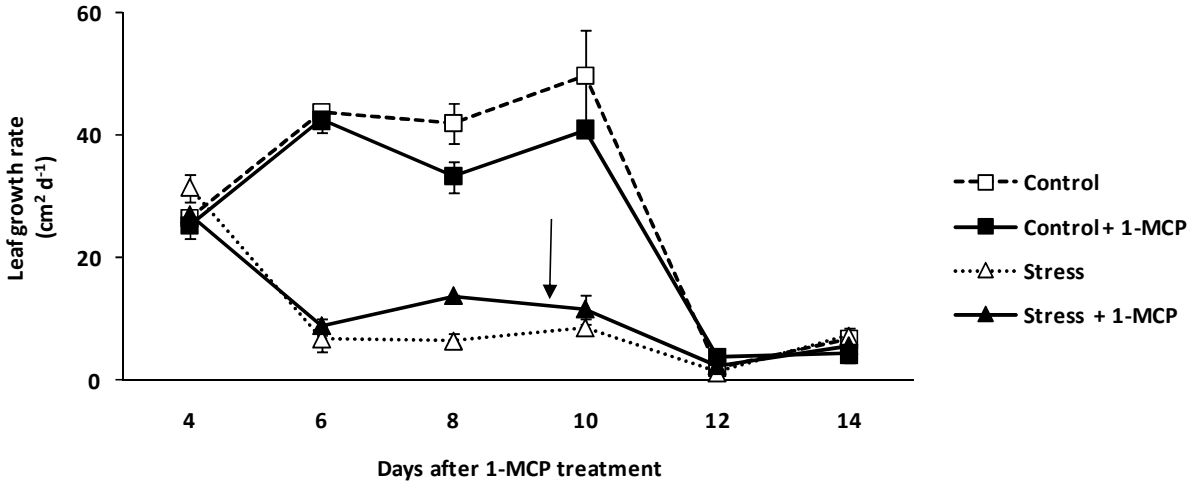

Fig. II.2. Leaf growth rate of well-watered (control, and control plus 1-MCP) and water-stressed (stress, and stress plus 1-MCP) cotton plants in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009. The arrow denotes when watering of stressed plants was initiated with the amount of water necessary to bring pots slightly above 8% MCW. This occurred on the evening of 9 DAT and was resupplied again whenever plants reached water status below the wilting point. Plants were thus kept at a constant level of stress. Bars represent SE where greater than the symbol.

Ethylene Emissions

Water deficit stress was imposed by terminating watering half of the pots following the gaseous treatment application with 1-MCP. Individual detached leaves of plants subjected to termination of daily irrigation with or without 1-MCP were assessed for ethylene production around peak, midday (Jasoni et al., 2002). Independent of 1-MCP treatment, water-stressed plants gradually started to appear wilted as the experiments progressed and the water transpired was lower (data not shown) than that of control plants. At 1, 5, 7, 9, 11, and 13 d after 1-MCP treatment, ethylene production levels of water-stressed plants were never above the control (Fig. II.3). Morgan et al. (1990) observed similar findings while assaying ethylene production of intact cotton plants subjected to gradual water stress imposed by cessation of irrigation. When treated with 1-MCP, ethylene rates of stressed plants exceeded those of the control at 1, 3, and 7 DAT. After 7 DAT, ethylene production dropped to levels below the control until 13 DAT. On the other hand, when control plants were treated with 1-MCP, ethylene production had a transient increase at 1 DAT, then did not go above the untreated control until 7 DAT; after that point in time, ethylene emissions were higher in comparison with the untreated control. It was evident that both the control and stressed plants when treated with 1-MCP changed their patterns of ethylene production after 7 DAT: plants that previously exceeded the control in terms of ethylene emitted became lower and those that were lower, started to be higher than the control. This observation may suggest the need of an additional 1-MCP application every 7 d to maintain a critical concentration for its activity in plants. Not taking into consideration the water regime,

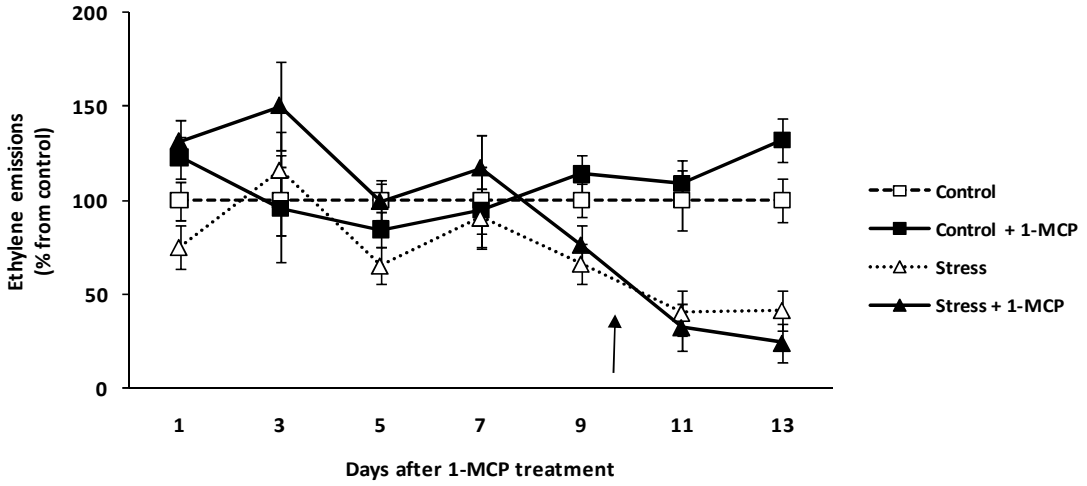

Fig. II.3. Ethylene emission of well-watered (control, and control plus 1-MCP) and water-stressed (stress, and stress plus 1-MCP) cotton plants in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009. The arrow denotes when watering of stressed plants was initiated with the amount of water necessary to bring pots slightly above 8% MCW. This occurred on the evening of 9 DAT and was resupplied again whenever plants reached water status below the wilting point. Plants were thus kept at a constant level of stress. Bars represent SE where greater than the symbol.

ethylene synthesis from both 1-MCP treatments exceeded the control at 1 DAT (Fig. II.3). These observed short-lived increases in ethylene synthesis following 1-MCP application is in agreement with de Wild et al. (2003) who reported stimulation of ethylene production by 1-MCP in freshly harvested pears while investigating CO_2 effects on ethylene synthesis. They concluded that this increase was due to the direct action of 1-MCP on the auto-inhibition phase of ethylene production by ethylene (preclimacteric stage) itself. While blocking the ethylene auto-inhibition phase, 1-MCP caused a transient climacteric stage (ethylene synthesis increase) in cotton leaves. This auto-inhibition (preclimacteric) stage reported by de Wild et al. (2003) referred to system 1 ethylene, which is the first stage in the ethylene production characterized with a basal low rate of ethylene synthesis (McMurchie et al., 1972). Negative-feedback regulated genes are involved in system 1, and cause an auto-inhibitory ethylene production. On the other hand, positive feedback regulated genes are present in system 2 leading to a auto-stimulatory ethylene production (Barry et al., 2000; Nakatsuka et al., 1998), climacteric stage (de Wild et al., 2003).

On the following day (3 DAT), well-watered plus 1-MCP treated plants had passed the transient peak of ethylene stimulation, while water-stressed plus 1-MCP treated plants were again exhibiting an increase in ethylene production above the control. This ethylene increase observed at 3 DAT only in stressed but not in well-watered plants plus 1-MCP was due to an intermediate water potential reached by stressed plants, leaf ψ_w = -1.4 MPa. While investigating ethylene emissions in intact cotton plants under drought conditions, Morgan et al. (1990) reported that water-stressed cotton plants did

not produce an increase in ethylene levels above the control, except when these plants reached water deficits between -1.4 and -1.6 MPa, corroborating with present findings.

Independent of 1-MCP, the stress treatments exhibited identical patterns starting at 7 DAT when both continuously reduced ethylene production until the last day plants were assayed for ethylene (Fig. II.3). These ethylene emission patterns precisely followed the drop in plant water potential (ψ_w). The steep drop in ethylene levels observed among stress treatments was slightly ameliorated after stressed plants were rewatered for the first time at 9 DAT, with sufficient moisture to keep them above permanent wilting point. Neither the stressed nor the stressed plus 1-MCP treatments showed an ethylene promotion during severe water deficit stress (Figs. II.3 and II.4), after 7 DAT (Fig. II.3). Morgan et al. (1990) reported that neither intact cotton nor bean plants 'demonstrated a promotion of ethylene release during severe stress'. Beltrano et al. (1997) reported that ethylene production of wheat ears of plants under water stress decreased and almost ceased gradually after plant ψ_w reached the value of -1 MPa. Beltrano et al. (1997) speculated that this decrease in ethylene production was due to cytosol dehydration that caused changes in protein (enzymes) conformation impeding these enzymes to synthesize ethylene.

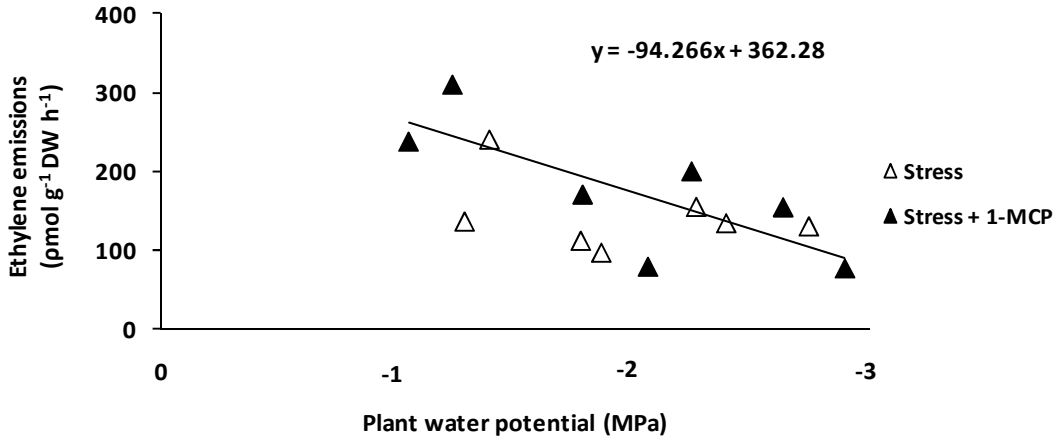

Fig. II.4. Ethylene emission as impacted by plant water potential of well-watered (control, and control plus 1-MCP) and water-stressed (stress, and stress plus 1-MCP) cotton plants in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009.

When ethylene emission was plotted against plant ψ_w, it was observed that independent of 1-MCP application, the control plants produced around 200 ρmol g^{-1} DW h^{-1} when plant ψ_w ranged between -1.0 and -1.3 MPa (data not shown). According to Hake et al. (1996) cotton plants are considered to be under water deficit stress when leaf ψ_w exceeds -1.8 to -2.0 MPa, with -1.8 MPa considered a mild stress (Griffiths and Parry, 2002). Ethylene levels of the stress treated plants decreased from 240 to 96 ρmol g^{-1} DW h^{-1} as the plant ψ_w decreased from -1.2 to -2.7 MPa. The stress plus 1-MCP treatment reduced ethylene levels from 309 to 75 ρmol g^{-1} DW h^{-1} as the plant ψ_w decreased from -1.0 to -2.9 MPa (Fig. II.4). Plants from both stress treatments (with and without 1-MCP) exhibited a decrease in ethylene emissions as plant ψ_w became progressively more negative (Fig. II.4). A linear relationship between plant ψ_w and ethylene production (Fig. II.4) became evident after 7 DAT (Fig. II.3). The lowest plant ψ_w examined generated the lowest ethylene emission of 75 ρmol g^{-1} DW h^{-1} (Fig. II.4), which was detected at 13 DAT in the stress plus 1-MCP treated plants (Fig. II.3). These findings are supported by previous work of Morgan et al. (1990)which showed a linear relationship between ethylene production and plant ψ_w, with the lowest ethylene production rate occurring at the lowest plant ψ_w, -2.9 MPa.

One of the main goals of this study was to determine if drought stress which was imposed slowly to mimic natural field drying would affect ethylene synthesis rates. For this purpose detached leaves from cotton plants that were subjected to water deficit stress under greenhouse conditions during the peak reproductive phase were used for ethylene quantification. As mentioned previously, many reports that detected an increase

in ethylene synthesis by water stress were conducted with detached plant parts (Adato and Gazit, 1974; Aharoni, 1978; Apelbaum and Yang, 1981; Ben-Yehoshua and Aloni, 1974; El-Beltagy and Hall, 1974; Graves and Gladon, 1985; Guinn, 1976; Hoffman et al., 1983; McKeon et al., 1982; Wright, 1981). When intact whole plants subjected to drought were assayed for ethylene synthesis by Hubick et al. (1986), and Morgan et al. (1990), it was reported that water deficit stress did not promote ethylene synthesis.

In the current study, assays of ethylene production from detached leaves of cotton plants experiencing water deficit stress showed that drought not only affected but it in fact decreased ethylene synthesis, revealing similar results to previous whole plant studies (Hubick et al., 1986; Morgan et al., 1990). Morgan et al. (1990) examined intact plants of rose (*Rosa hybrida* L., cv Bluesette), beans (*Phaseolus vulgaris* L.), and cotton, as well as detached leaves of cotton plants for levels of ethylene production under water stress. These assays for ethylene synthesis were made from plants or plant parts enclosed in air flow cuvettes. None of the species showed promotion in ethylene synthesis compared with a non-water stressed control. However, in the same paper they reported that leaves of non-stressed plants (beans and cotton) when air dried produced increases in ethylene synthesis above the control.

In some instances, when whole plants were subjected to some kind of treatment that induced water deficit stress, and then plant parts were detached and evaluated for ethylene production, ethylene synthesis was reported to be increased (El-Beltagy and Hall, 1974; Graves and Gladon, 1985; Guinn, 1976). The approach of Graves and Gladon (1985) consisted of exposure of whole plants during 48 h to polyethylene glycol

(PEG), which was used to induce drought quickly, constituting an unrealistic environment of what plants would have experienced naturally in the field under a natural drought cycle that is imposed slowly. Guinn's (1976) technique to impose drought in the greenhouse consisted of a more realistic approach since he discontinued watering the plants. However, he imposed detached young bolls (1.5 to 4.5 d old after anthesis) to quick desiccation by sealing them in an airtight chamber with silica gel during 2 to 24 h prior to ethylene evaluation. The only similarities between these works (Graves and Gladon, 1985; Guinn, 1976) and the current study consist on the fact that all imposed a water deficit-inducing treatment to whole plants and plant parts were detached for ethylene production. The fact that these approaches (El-Beltagy and Hall, 1974; Graves and Gladon, 1985; Guinn, 1976) detected an ethylene production increase, while the current study which collected ethylene from blades of the uppermost unfurled leaf of stressed plants that were subjected to a slow and more realistic drying period did not detect an ethylene increase, suggests interaction between how quick drought is imposed and ethylene synthesis. The approach of El-Beltagy and Hall (1974) was similar to the present research since plants were exposed to drying soil and detached leaves were incubated and sampled for ethylene. However, the two presented diverging outputs, since El-Beltagy and Hall (1974) reported an increase in ethylene due to drought. Moreover, El-Beltagy and Hall (1974) did not mention if there was consistency in the position occupied by the leaves sampled based on plant architecture. If there was sampling inconsistency, this may have been one source of diverging output compared to the approach of this current study, since Morgan et al.(1992) revealed that leaves

synthesize different amounts of ethylene based on their distribution within the plant mainstem.

Ethylene Responsive Cotton Genes

Normalized expression of genes associated with ethylene synthesis was investigated at 1, 7, and 13 DAT since these were key days for observed ethylene emission during the studies. To minimize interference of destructive sampling to either ethylene emission or ethylene related gene expression, samples for each study were collected from different plants grown in the same pot. Even though samples were originated from different plants, trend similarities were still observed between ethylene production and gene expression (Fig. II.5). Gene *ACS6*, which encodes for enzymes that convert SAM to ACC (precursor of ethylene), of the stress plus 1-MCP treatment showed higher expression than that of control levels at 1 and 7 DAT, and decreased in expression below the control at 13 DAT (Fig. II.5 B). Ethylene emission of this treatment followed the same trend, showing higher emissions than the control at 1 and 7 DAT, and below the control at 13 DAT (Fig. II.5 A). The control plus 1-MCP treatment also showed similarities between ethylene levels and *ACS6* expression (Fig. II.5), with the ethylene

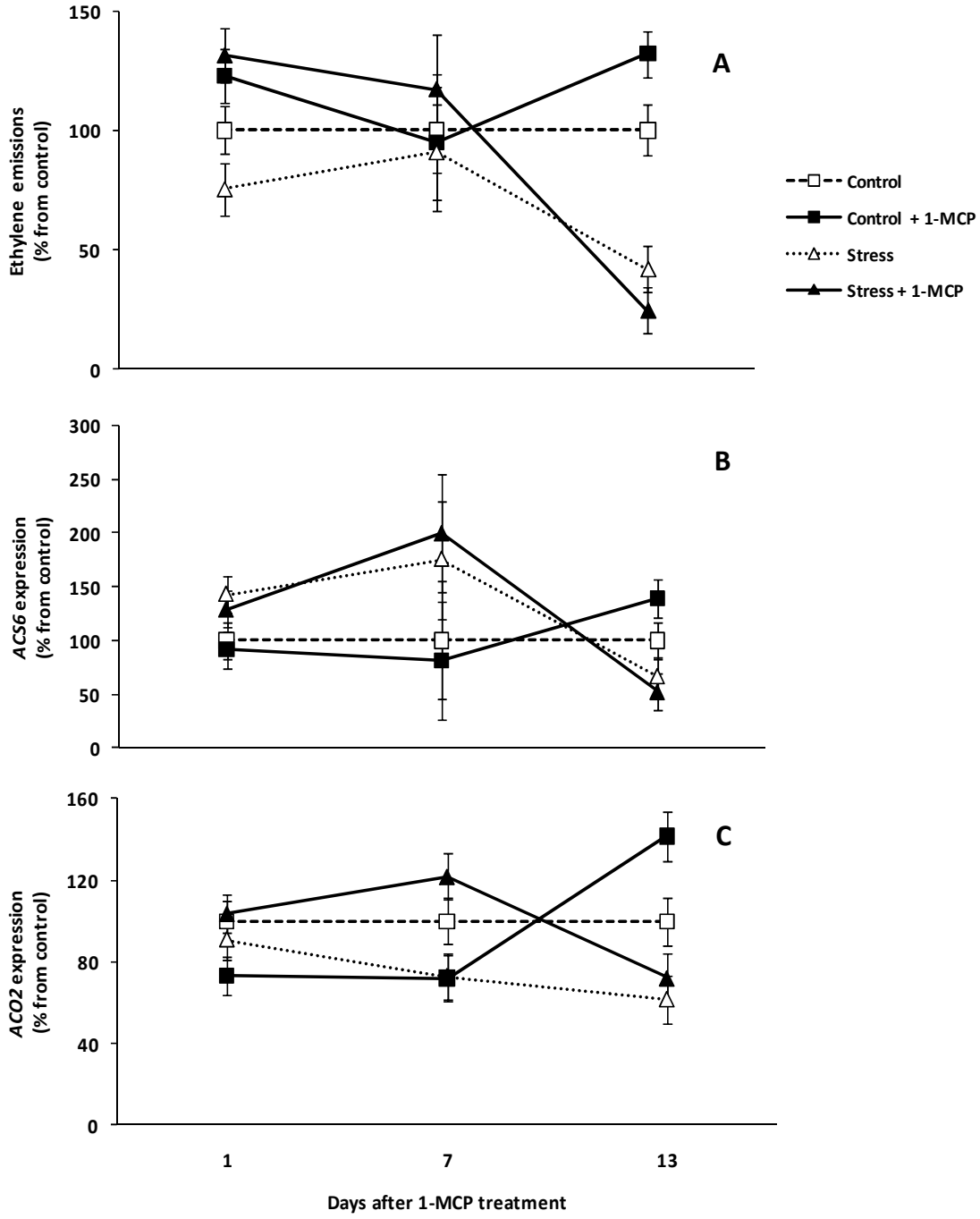

Fig. II.5. Ethylene emissions and *ACS6, ACO2, ETR5,* and *GDSL* cotton gene expression of well-watered (control, and control plus 1-MCP) and water-stressed (stress, and stress plus 1-MCP) cotton plants in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009. Bars represent SE where greater than the symbol.

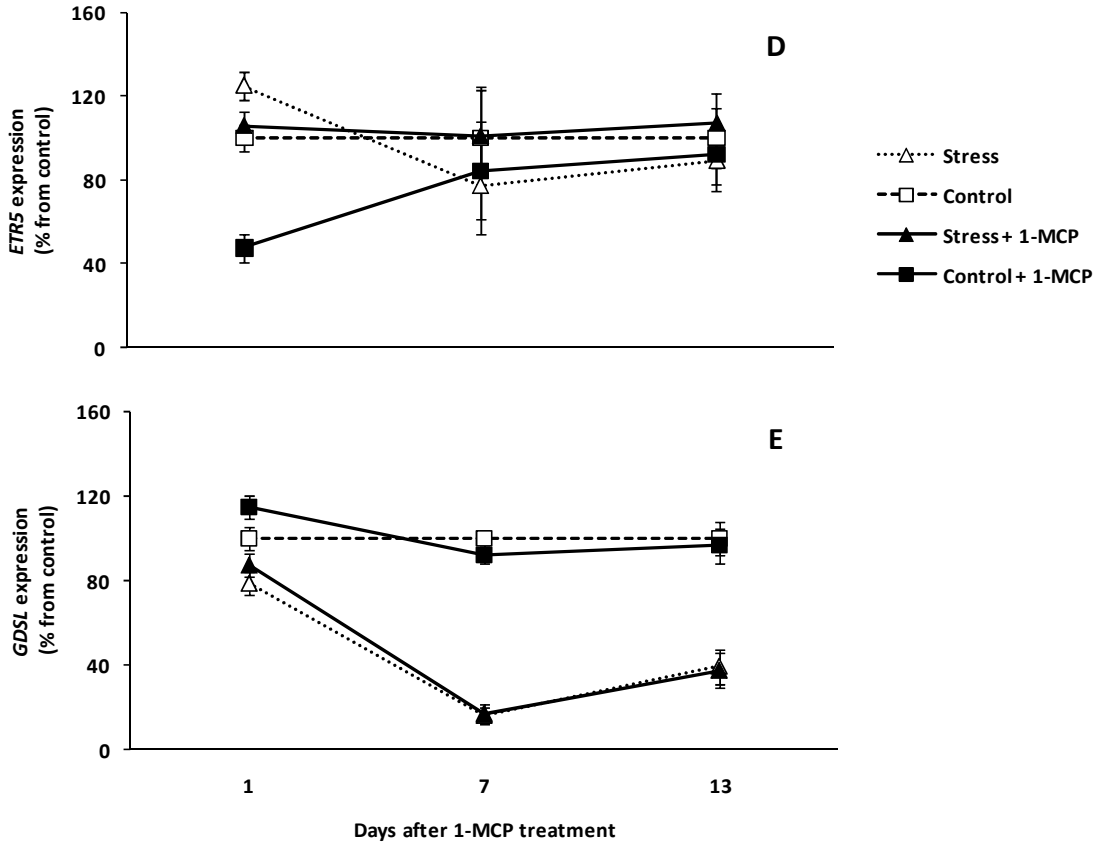

Fig. II.5 continued.

emissions and the expression lower than the control at 7 DAT and above it at 13 DAT (Fig. II.5 A and B). *ACS6* expression and ethylene production of stressed plants were only similar at 13 DAT, when both remained lower than the control (Fig. II.5 A and B), indicating down-regulation of the gene by stress and consequently low ethylene emissions. Water regime was a key factor for *ACS6* expression and significantly influenced its regulation at 1, 7, and 13 DAT, while 1-MCP seemed not to affect its expression (Table II.1).

An enzyme that converts ACC to ethylene is encoded by *ACO2*. Unlike *ACS6* expression, water stress down-regulated the expression of *ACO2* throughout the whole experiment, with its expression being lower than the control (Fig. II.5 C). Likewise, ethylene synthesis showed the same trend as *ACO2* and was repressed by water stress throughout the studies (Fig. II.5 A), which reflected the important role played by ACO during ethylene synthesis. The importance of ACO observed in this study coincides with the results published by Dunlap and Robacker (1994). These researchers concluded that ethylene production in muskmelon tissues was determined by the ability of ACO

Table II.1. Analysis of variance for *ACS6, ACO2, ETR5,* and *GDSL* cotton gene expression of cotton plants in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009.

	Normalized gene expression (x 10^{-7})			
Source	*ACS6*	*ACO2*	*ETR5*	*GDSL*
	1 d after 1-MCP treatment			
1-MCP application (M)				
No 1-MCP	9	0.31	208	1.2
1-MCP	9	0.29	142	1.3
Water regime (W)				
Water-stressed	11	0.31	214	1.1
Well-watered	7	0.28	136	1.4
ANOVA		*P > F*		
M	0.5152	0.4787	<.0001	0.0440
W	0.0303	0.2820	<.0001	0.0003
M x W	0.8841	0.0524	0.0220	0.5658
	7 d after 1-MCP treatment			
1-MCP application (M)				
No 1-MCP	11	0.29	92	1.0
1-MCP	11	0.32	96	0.9
Water regime (W)				
Water-stressed	14	0.32	92	0.3
Well-watered	7	0.28	96	1.6
ANOVA		*P > F*		
M	0.9617	0.3650	0.8669	0.4231
W	0.0967	0.3388	0.8974	<.0001
M x W	0.6985	0.0035	0.4151	0.2803
	13 d after 1-MCP treatment			
1-MCP application (M)				
No 1-MCP	15	0.80	299	1.1
1-MCP	18	1.06	315	1.1
Water regime (W)				
Water-stressed	11	0.66	311	0.6
Well-watered	22	1.19	304	1.6
ANOVA		*P > F*		
M	0.4714	0.0419	0.7313	0.7557
W	0.0033	0.0003	0.8866	<.0001
M x W	0.1505	0.2098	0.3936	0.9133

enzymes to convert ACC to ethylene, rather than on ACC presence or the abundance of ACC. At 7 and 13 DAT, ethylene synthesis followed *ACO2* expression in the control plus 1-MCP and stress plus 1-MCP treatments (Fig. II.5 A and C). The *ACO2* in the control plus 1-MCP treatment was down-regulated at 7 DAT, then up-regulated at 13 DAT when compared to the untreated control. However, stress plus 1-MCP treatment showed opposite behavior: where *ACO2* was up then down-regulated at 7 and 13 DAT, respectively (Fig. II.5 C). Investigations of the main effects revealed that 1-MCP and water stress significantly affected *ACO2* expression at 13 DAT (Table II.1). *ACO2* expression was up-regulated by 1-MCP and down-regulated by water stress.

At 1 DAT, *ETR5* which encodes for an ethylene receptor in the signal transduction pathway was up-regulated in plants under stress (Fig. II.5 D). Since gene expression only quantifies mRNA concentration in a sample, it is impossible to predict the real outcome of this result. If the mRNA quantified resulted in an increase in the functional amount of ETR5 receptor protein in plant tissues, this would have made these tissues less susceptible to the same level of ethylene. However, if there was a simultaneous increase in the turnover of existing ETR5 protein that had already been bound to stress-induced ethylene, this would have resulted in a steady-state level of ETR5 protein with a resetting of the tissues to a base level of sensitivity. On the other hand, water regime significantly interacted with 1-MCP application (Table II.1). As a result, *ETR5* was down-regulated by 1-MCP under well-watered conditions (Fig. II.5 D), suggesting that if mRNA resulted in a decrease in the functional amount of ETR5 protein in the tissues, they would have been more susceptible to the same level of ethylene. However, if there

was a simultaneous decrease in the turnover of existing ETR5 protein previously bound to stress-induced ethylene, this would have resulted in a declining level of unbound ETR5 protein thus setting these tissues to a high level of sensitivity.

Researchers observed that *GDSL* was very responsive to ethylene. The relationship between GDSL-motif lipase gene, which encodes for multifunctional property enzymes, and ethylene is still not understood (S. Finlayson, personal communication). Independent of 1-MCP, *GDSL* was highly sensitive to drought and was progressively down-regulated up to 7 DAT (Fig. II.5 E). From 7 to 13 DAT, the slope of its expression changed reflecting the point at which stressed plants were resupplied with water lost by evapotranspiration (9 DAT). During the entirety of gene expression evaluations, GDSL was significantly down-regulated by water deficit stress (Table II.1); consequently, *GDSL* may have potential for not only an ethylene-responsive gene but also a drought-responsive gene. The 1-MCP compound only showed a significant effect on *GDSL* expression at 1 DAT (Table II.1).

CONCLUSIONS

The results of this study indicated that ethylene synthesis had a linear relationship with plant ψ_w status. Water deficit caused a continuous decrease in ethylene synthesis, and as drought progressed ethylene reached the lowest rate of 75 ρmol g^{-1} DW h^{-1} at -2.9 MPa. Short-lived increases in ethylene synthesis were observed following 1-MCP applications at 1 DAT. 1-MCP caused a transient climacteric stage (ethylene synthesis increase) in cotton leaves, while blocking the ethylene auto-inhibition phase.

The observed detrimental effect of drought on ethylene levels was partially validated by the expression of *ACS6* and *ACO2* cotton genes. 1-MCP significantly down-regulated the expression of *ETR5*, and appeared to have altered ethylene perception 1 d after 1-MCP application. The *GDSL* cotton gene showed potential as a drought-responsive gene. 1-MCP showed little influence on ethylene synthesis and expression of related genes. Leaf growth was affected by 1-MCP only at one day, while water deficit stress inhibited it throughout the studies.

CHAPTER III

DROUGHT EFFECTS ON GAS EXCHANGE, CHLOROPHYLL, AND PLANT GROWTH OF 1-MCP TREATED COTTON PLANTS

OVERVIEW

Drought impacts cotton (*Gossypium hirsutum* L.), affecting its physiological, morphological, and agronomic parameters. Water stress causes cotton plants to reduce boll production, and increase abortion of young fruit. Ethylene plays an important role in abscission; thus, it is desirable to prevent fruit loss induced by ethylene prior to abscission. Ethylene inhibitors, such as 1-methylcyclopropene (1-MCP), are an alternative to cope with the loss of reproductive structures. The objective of this study was to examine the effects of 1-MCP on gas exchange, plant growth/development and yield components of cotton plants under drought stress during the reproductive phase. A 2-yr greenhouse study was conducted as a 2x2 factorial design in a split-block arrangement with five replications. Treatments were two 1-MCP rates (0 and 2.4 g a.i. L^{-1}) during a 14-h overnight incubation that were then subjected to two water regimes (control and stressed). Gas exchange analysis revealed that water deficit stress started to impact plants at a moderate water stress, 5 DAT (-1.4 MPa). The 1-MCP increased water use efficiency in well-watered plants at 1 DAT. Many of the yield components, plant mapping, and biomass parameters investigated were adversely affected by drought. However, drought increased specific leaf weight, chlorophyll content, and harvest index. The 1-MCP improved reproductive node numbers mainly during drought, but did not lead to a better harvest index, since 1-MCP caused high abscission. In conclusion, 1-

MCP did not ameliorate any of the detrimental effects of water stress on gas exchange when water stress started to impact cotton plants. 1-MCP had little or no positive effect on plant mapping, dry matter partitioning and chlorophyll content.

INTRODUCTION

Several variables compose lint cotton (*Gossypium hirsutum* L.) yield. In terms of significance to lint yield, these variables are the number of bolls m^{-2}, and within-boll yield parameters such as seed per boll, and lint per seed (Worley et al., 1974). A study in multiple locations and with several cultivars in Australia (Kilby, 2005) showed that lint yield was strongly correlated with boll m^{-2}, followed close by seed per boll and lint per seed. During an 11-yr study in Louisiana, Boquet et al. (2004) also observed that bolls m^{-2} was the most significant variable to lint yield. While evaluating boll retention over 188 upland cotton inbred lines, Wu et al. (2005) reported that the number of bolls is one of the most important yield components. These recent studies corroborate to earlier findings by Worley et al. (1974). Thus, these studies (Boquet et al., 2004; Kilby, 2005; Worley et al., 1974; Wu et al., 2005) collectively concluded that the variable which contributes the most to lint yield is the number of bolls m^{-2}. However, these yield components are strongly affected by water deficit stress, since water deficit is one of the main limiting factors for cotton (Gerik et al., 1996) and other crops cultivated worldwide (Pettigrew, 2004a).

Water deficit stress detrimentally impacts cotton production (Howell et al., 2004; Mooney et al., 1991; Pettigrew, 2004b). Although cotton is able to maintain a leaf turgor potential (ψ_t) by osmotic adjustment under moisture deficit, it eventually faces a

reduction in leaf water potential (ψ_{wl}) under dry conditions (Ball et al., 1994; Nepomuceno et al., 1998; Turner et al., 1986). In response to drought, stomata tend to close reducing leaf conductance that ultimately affects leaf photosynthesis (Ephrath et al., 1990; Faver et al., 1996; Genty et al., 1987). Under water stress, overall dry matter accumulation in cotton plants is decreased (Mooney et al., 1991), expansion of leaf blades and plant growth is reduced, thereby promoting stunted growth (Ball et al., 1994; Gerik et al., 1996). Limited water availability also causes cotton plants to generate fewer flowers, which consequently reduces boll production. Under severe stress during reproductive development boll abortion is increased, thus reducing lint yield (Gerik et al., 1996; Pettigrew, 2004a; Turner et al., 1986). Since the number of bolls m^{-2} contributes the most to cotton lint yield (Boquet et al., 2004; Kilby, 2005; Worley et al., 1974; Wu et al., 2005), alternatives to reduce or prevent abortion of cotton bolls due to water deficit stress are desirable. Morgan et al. (1992) observed a burst in ethylene levels that lasted 4 days prior to abscission. The authors concluded that this peak in ethylene may have been the necessary signal to initiate cell wall hydrolysis in the abscission zone followed by abscission. Since ethylene plays an important role in abscission (Guinn, 1976; Morgan et al., 1992; Steel and Torrie, 1980), it is desirable to protect yield by preventing fruit loss induced by a peak in ethylene synthesis prior to abscission. Preventing loss of flowers and young fruit is essential to enhance cotton yield (Heitholt et al., 1993), and ethylene inhibitors offer a potential alternative to cope with the loss of reproductive structures, and thereby, enhance cotton yield.

The compound 1-methylcyclopropene (1-MCP) is a gaseous ethylene antagonist that blocks ethylene receptors, consequently inhibiting its perception and preventing ethylene effects in the plant tissues (Blankenship and Dole, 2003; Sisler and Serek, 1997). The affinity of 1-MCP to ethylene receptors is approximately 10x greater than the affinity of ethylene to its receptors (Blankenship and Dole, 2003). The compound is widely used in horticultural products (Fan and Mattheis, 2000). Previous studies in horticulture have mainly focused on its use in post-harvest physiology of climacteric fruit to counter the detrimental effects of ethylene. These studies show that the compound impacts a variety of physiological processes, such as decreasing ethylene synthesis (Blankenship and Dole, 2003; Dong et al., 2001; Jeong et al., 2002), respiration (Blankenship and Dole, 2003; Dong et al., 2001; Fan and Mattheis, 2000), and chlorophyll degradation (Blankenship and Dole, 2003; Fan and Mattheis, 2000; Jiang et al., 2002), thus extending the shelf-life of climacteric fruit (Fan and Mattheis, 2000).

The primary objective of this study was to establish how drought affects gas exchange, plant growth/development and yield components of 1-MCP treated cotton plants during the peak of their reproductive phase under greenhouse conditions. A secondary objective was to determine if gas exchange, plant growth/development and yield component responses to drought could be altered by the presence of 1-MCP treatment.

MATERIALS AND METHODS

Growth Conditions and Plant Material

Plants were grown in the Borlaug Center greenhouses at Texas A&M University. Growth conditions consisted of a day/night temperature of 32/31 °C in 2008, and 27/26 °C in 2009, with a day/night relative humidity of 57/55% in 2008, and 41/39% in 2009. Measurements were made with a Center 315 Temperature and Humidity Meter (Center Technology Corp., Taiwan). Midday photosynthetic photon flux density (PPFD) was measured with the quantum sensor of a Li-Cor 6400 XT infrared gas analyzer (LI-COR Inc., Lincoln, NE) during gas exchange measurements, and averaged 900 and 850 μmol m^{-2} s^{-1} in 2008 and 2009, respectively. Plants were provided with the natural photoperiod of the locality (College Station, TX: 30°30'26.74" N, 90°20'58.83" W).

Fritted clay which is sold as Absorb-N-Dry (Balcones Mineral Corp., Flatonia, TX) was chosen as a medium for growing plants. This medium is known for its quick drainage and low dry-bulk density, but mainly for its capacity of holding a large quantity of plant-available water (van Bavel et al., 1978). These properties were especially important as the medium was intended to impose a gradual dry down period as a source of moisture deficit treatment. Cotton seeds (*Gossypium hirsutum* L. cv. 'Americot NexGen 2448 R') were placed in plastic pots with the following dimensions: height 0.28 m, top width 0.30 m, and 15 L capacity. These pots were filled with 11.5 L of fritted clay. After emergence, seedlings were thinned to two uniform plants per pot, with each plant located on opposite sides of the pot. Plants were watered thoroughly daily with reverse osmosis (RO) water with an electro-conductivity of 6.7 μS, and fertilized with

≈1L pot^{-1} of a 0.7% (w/v) solution of 20-20-20 (%N, P and K as N, P_2O_5 and K_2O equivalents) fertilizer plus micronutrients (Miller Greenhouse Special, Miller Chemical and Fertilizer Co. Corp., Hanover, PA). Fertilization was suspended when treatments were initiated to avoid confounding of data.

Application of Treatments and Experimental Design

Two studies were performed, one during the spring of 2008 and the second during the spring of 2009. These studies consisted of four treatments with five replicates. Treatments were commenced when plants averaged 16 mainstem nodes, which coincided with mid-bloom. After the potting medium was brought to container capacity, 140 pots out of 200 were randomly chosen and moved to the potting room of the greenhouse facility and placed into 4 m^3 sealed, polyethylene tents. These tents were equipped with a SmartFresh^{SM} Research Tablet Generator system (AgroFresh Inc., PA) in which 1-MCP tablets were placed to generate 1-MCP as a gas. A Coleman tent fan (The Coleman Company, Inc., KS) was used to uniformly deliver the gaseous treatment. This generation/distribution system was placed in the middle of the tents in order to guarantee a better distribution of the gas. Half of the pots (70) placed in these tents were randomly assigned to no 1-MCP and the other half was exposed to 2.4 g a.i. L^{-1} of air. Pots were kept inside the tents for 14 h overnight since a previous report of Jeong et al. (2002) showed that a gaseous application of 1-MCP with a treatment duration of less than 12 h did not provide sufficient protection. Early next day the pots were transported back to the greenhouse tables, and randomly arranged as a split-block in a 2x2 factorial experiment. The split was the two watering regimes to which 70 pots were subjected to

daily irrigation at container capacity, and the other 70 were subjected to a drying period where water was withheld. This allowed these pots to dry slowly (mimicking natural field conditions) until plants reached the wilting point (previously determined) for the first time. After that, water stressed plants were supplied with sufficient water to be slightly above the wilting point. These plants were re-supplied again whenever plants reached a water status below wilting point to keep plants at a constant water deficit stress during the 22 d after 1-MCP treatments were initiated. The magnitude of the drought treatments was determined by a preliminary experiment in which pots were watered to saturation and allowed to drain. One hour later, after plants had attained constant weight, they were weighed to determine the container capacity for holding water. Two plants (stems and roots) were pulled from a random pot and weighed. The fresh weight of these two plants was subtracted from the container capacity to determine the medium container capacity weight (MCW) by itself. At wilting, weight of the water-depleted medium was 8% of the MCW. Fifteen randomly picked extra pots were assigned to drought stress for use in monitoring the amount of water lost by evapotranspiration. Every day, these pots were weighed, averaged, and subtracted from the weight of 2 random plants (fresh weight; roots and shoots) in order to assess the MCW. When the container capacity weight reached values below 8% MCW (meaning the stressed plants were at wilting), the amount of water necessary to bring pots slightly above 8% MCW was supplied to these 15 extra pots and also to all the other 70 pots under water stress treatment., This preliminary procedure for determination of wilting point and evapotranspiration was adapted from Starman and Lombardini (2006). Watering took

place at night, except at 1 DAT. On this day well-watered plants were watered as soon as they were moved back to the greenhouse, which was at least 1 h prior to any readings or measurements.

Sampling Procedure

Pots subjected to well-watered conditions were irrigated thoroughly in the morning. This was completed daily at least 1 h prior to any sampling and/or measurements. Measurements were initiated at day one, which was the same day pots were subjected to water regime treatments following their return to the greenhouse. This timing also coincided with one day after 1-MCP treatments (DAT) were initiated. Measurements/samplings were taken every other day on 20 pots; 5 pots replicates were used for each treatment. A new set of 20 pots was used on the next evaluation day.

Water Status Assessments

A HH2 Theta Probe (Delta-T Devices, Cambridge, UK) set on the mineral soil setting was used to determine pot volumetric water content of the upper 6 cm of medium (Starman and Lombardini, 2006). While pot volumetric water content was being determined, pots were simultaneously weighed for a gravimetric evaluation of the soil-water status. These gravimetric and probing measurements took place daily for the 15 extra pots that were used to monitor early evening water loss, as well as gas exchange (1100 to 1400 h) every other day throughout the experiment. Leaf water potential (ψ_{wl}) determination took place after gas exchange measurements using the procedure of Scholander et al. (1965). This determination of water potential was made only for the set of 20 pots assigned for that particular day by using a pressure chamber, in which the

third uppermost fully-expanded leaf from one of the plants per each pot was placed. Leaves were placed into the chamber within 15 s of excision and the chamber was pressurized at a rate of 0.02 MPa s^{-1} as previously reported by Turner (1988).

Gas Exchange Evaluations

Gas exchange measurements were conducted between 1100 and 1400 h during 14 d on every other day after treatments were initiated. During the warm-up procedures for the Li-Cor 6400 XT (LI-COR Inc., Lincoln, NE), maximum midday PPFD was determined with the quantum sensor of the infrared gas analyzer. The same instrument was also used to measure the ambient greenhouse CO_2 level. Measurements of the third uppermost fully-expanded leaf (Patterson et al., 1977) from one of the plants in each of the pots were taken at maximum PPFD and ambient CO_2 concentration previously determined in order to mimic the same greenhouse environment in which the plants were located. This also guaranteed that all 20 plants assessed for a particular day had the same PPFD and CO_2 levels. Therefore, if differences were detected, they could be attributed to differences in treatment rather than differences in light intensity or CO_2 levels provided in the closed chamber. The maximum PPFD was supplied by a Red/Blue Light Source 6400-02B (LI-COR Inc., Lincoln, NE) on the adaxial surface of the chosen leaf. Leaf adaption to the light and CO_2 conditions inside the chamber was monitored by the instant displayed curves of CO_2 carbon assimilation and stomatal conductance, and each data point was only recorded after these curves were at steady state. The ability of leaf to quickly adapt to the instrument conditions decreased as water deficit stress increased and ranged from 15 s (well-watered plants) to 360 s (water-stressed plants). Measurements to

assess gas exchange characteristics as water deficit stress increased included CO_2 net assimilation rate (A), stomatal conductance (g_s), transpiration rate (E), intercellular CO_2 (C_i), leaf vapor pressure deficit (VPDL), leaf temperature, and instantaneous water use efficiency (WUE) which was calculated as described in Starman and Lombardini (2006).

Plant Mapping and Chlorophyll Content

Plants were assessed for fruit set and retention by plant mapping according to Landivar et al. (2010), and also for biomass production and chlorophyll content at 22 DAT and not at 14 DAT. This allowed the plants to experience the treatment effects over a longer period of time in order for their differences to become more evident. These late-season plant evaluations occurred at late-bloom stage when most of the canopy was developed. Plant mapping is a way to measure the plant status during its growth and development phases and is commonly used in cotton production evaluations. This procedure includes a variety of vegetative and reproductive measurements such as plant height, number of main-stem nodes, number and location of bolls and flowers, etc. (Jenkins and McCarty, 1995). Twenty-two days after the treatments were initiated, both plants in each pot were subjected to plant mapping. Plant height was measured from the surface of the potting media to the plant apex. The chlorophyll level of the third uppermost fully-expanded leaf from one of the plants per each pot was determined with a SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc., Osaka, Japan); the reading consisted of the average of three readings per each leaf following proper instrument calibration. Other determinations made at this time consisted of the plant mapping followed by biomass procedures.

Biomass Evaluation

Biomass procedures were completed on the same day as plant mapping and chlorophyll content evaluations using the same plants. Both plants per each pot were cut at the soil surface and separated into stems plus petioles (vegetative weight), squares plus flowers and bolls (reproductive weight), and leaves (Pettigrew, 2004b). Total leaf area readings were taken by using a Li-Cor 3100 leaf area meter (LI-COR Inc., Lincoln, NE), which when divided by the total leaf weight provided the specific leaf weight (SLW), according to Pettigrew (2004b). The samples were dried for 96 h at a minimum of 72 °C (Goldman et al., 1989; Sánchez-Blanco et al., 2009; Starman and Lombardini, 2006) and dry weights were determined gravimetrically.

Data Analysis

Neither 1-MCP application nor water regime interacted with years; thus, data were analyzed across years. Data were subjected to analysis of variance using PROC MIXED (Littell et al., 2006) of SAS version 9.2 (SAS Institute Inc., 2008) for a split-block design, where main plot was 1-MCP application, and subplot was water regime. Homogeneity of variance across years was tested for each variable. Paired *t*-tests were used to assess differences between two means. Multiple mean comparisons were made using Tukey's test at $P \leq 0.05$ (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Water Status Assessments

Soil water content (Fig. III.1 A) and leaf water potential (Fig. III.1 B) of well-watered treatments were kept close to container capacity throughout the experiment,

while water-stressed pots were allowed to dry gradually until they reached the wilting point, which occurred for the first time at 9 DAT. On that night, the amount of water necessary to bring the pots slightly above 8% MCW was supplied (indicated by arrow) only to pots under water stress treatment. This first irrigation of stressed plants was visualized by a discreet change in the slope of soil water content and leaf water potential (Fig. III.1 A and B), indicating that the pot medium and consequently plants regained water content only to eventually lose it again by evapotranspiration. There were no differences in soil water content or ψ_{wl} among well-watered treatments (control, and control plus 1-MCP), or among water-stressed treatments (stress, and stress plus 1-MCP). The only differences observed in soil water content and in ψ_{wl} were between well-watered and water-stressed treatments (Fig. III.1 A and B). The only significant differences in ψ_{wl} under well watered conditions were detected at 7 DAT, while under water-stress it was observed at 9 DAT. At both days, the 1-MCP caused a decrease in ψ_{wl} (Fig. III.1 B).

The ψ_{wl} of the well-watered treatments showed a gradual decrease as the experiment continued. It was speculated that this decrease in ψ_{wl} was due to the proximity of the pots to the greenhouse cooling system. As the days of evaluation progressed in this study, the tables that were measured were closer to the cooling system.

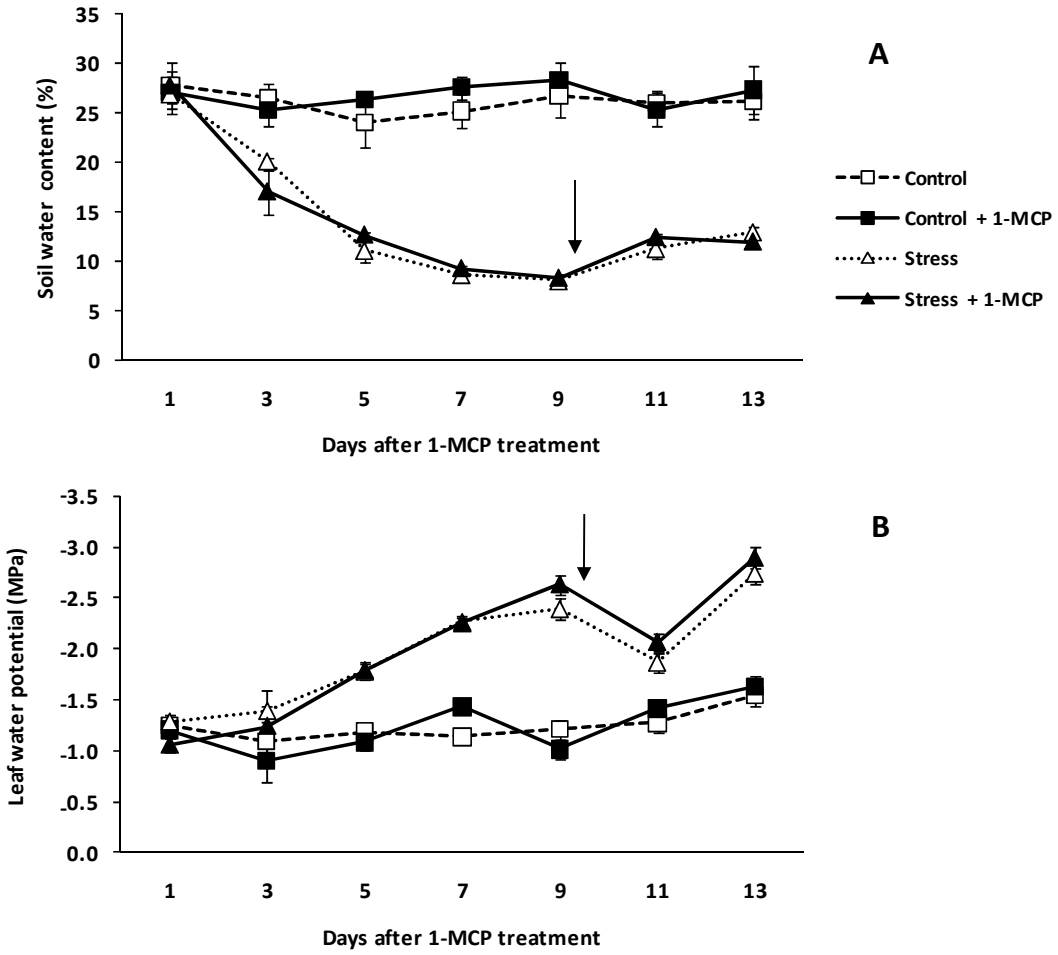

Fig. III.1. Soil water content (A), and leaf water potential (B) during the experiment of well-watered (control, and control plus 1-MCP) and water-stressed (stress, and stress plus 1-MCP) cotton plants in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009. The arrow denotes when watering of stressed plants was initiated with the amount of water necessary to bring pots slightly above 8% MCW. This occurred on the evening of 9 DAT and was resupplied again whenever plants reached water status below the wilting point. Plants were thus kept at a constant level of stress. Bars represent SE where greater than the symbol.

In other words, the table assigned to 1 DAT was the farthest from the cooling system, and the table designated to 13 DAT was the closest. These tables were positioned perpendicular to the cooling system to intentionally block this source of variability in the study. Thus, the closer plants were to the forced-air cooling system the faster the air moved, reducing the boundary layer of leaves and consequently causing a greater loss of moisture when compared to plants placed farther from this system.

Gas Exchange Evaluations

The CO_2 net assimilation (A) of plants that were well-watered had more carbon assimilation than water-stressed plants beginning at 5 DAT which continued to the end of the experiments (Table III.1 and Fig. III.2). Water-stressed plants at 5 DAT had a ψ_{wl} of -1.8 MPa, which is considered a moderate water stress according to Hake et al. (1996), that continued to decrease up to -2.9 MPa (Fig. III.1). Water stress reduced carbon assimilation up to 65% at 9 DAT when compared with plants under well-watered conditions (Table III.1). This study, similar to that of Ephrath et al. (1993), showed that leaf A was adversely affected by the increasing level of water stress, and showed an exponential relationship between A and ψ_{wl} (Fig. III.2 A). Carbon assimilation followed the same pattern as the photosynthetically active radiation (PAR); and independent of treatments, A showed abrupt drops at 1 and 5 DAT (Fig. III.2 B). These drops reflected changes in PAR levels, which were highly reduced due to overcast conditions on the day of the measurements. In Fig. III.2 A, the data points not fitting the curve were collected on these overcast days, and consequently had lower A values. There was no effect of

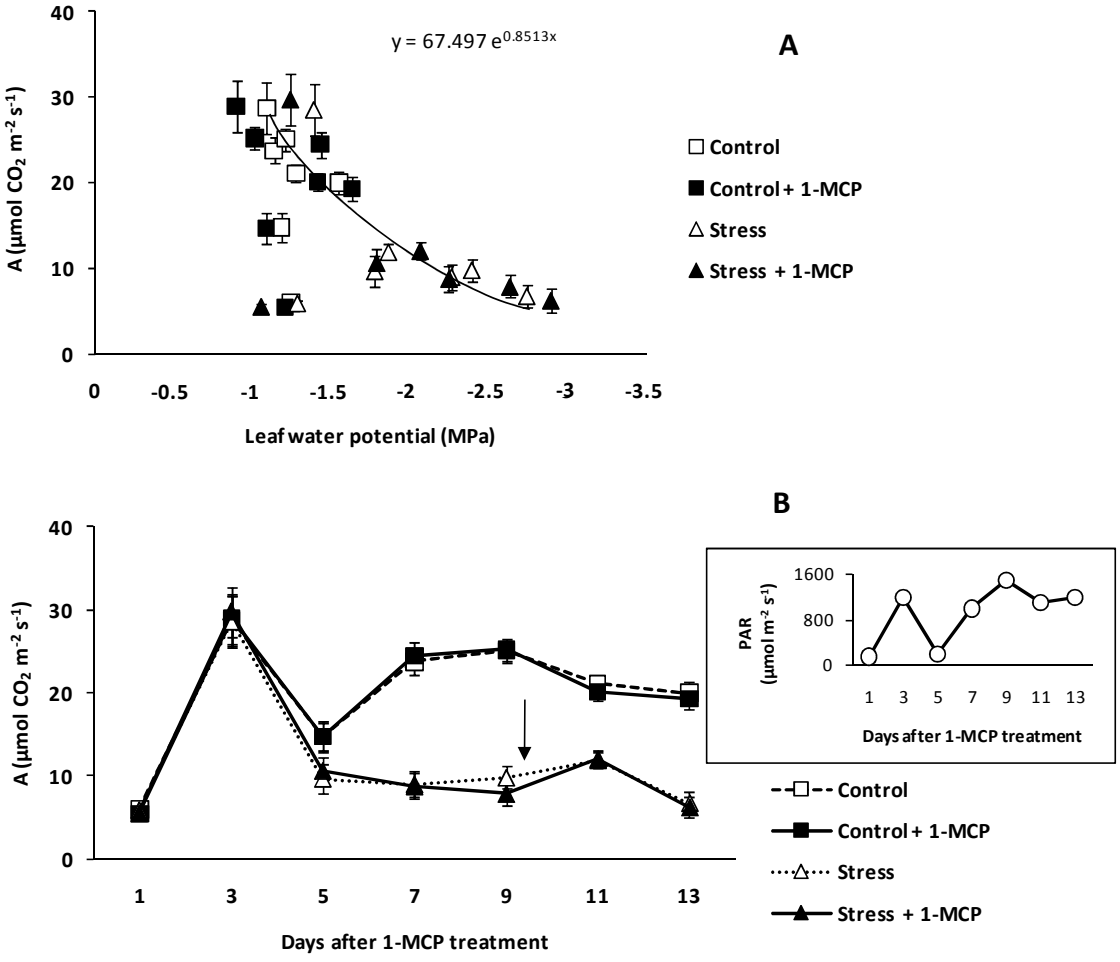

Fig. III.2. Relationship of CO_2 net carbon assimilation to leaf water potential (A), CO_2 net carbon assimilation and photosynthetically active radiation, PAR, during the experiment (B) for well-watered (control, and control plus 1-MCP) and water-stressed (stress, and stress plus 1-MCP) cotton plants in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009. The arrow denotes when watering of stressed plants was initiated with the amount of water necessary to bring pots slightly above 8% MCW. This occurred on the evening of 9 DAT and was resupplied again whenever plants reached water status below the wilting point. Plants were thus kept at a constant level of stress. Bars represent SE where greater than the symbol.

Table III.1. CO_2 net assimilation rate, stomatal conductance, and intercellular CO_2 from 1 to 13 days after 1-MCP treatments were initiated on cotton plants in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009.

	Days after 1-MCP treatment						
Source	1	3	5	7	9	11	13
	CO_2 net assimilation rate (μmol CO_2 m^{-2} s^{-1})						
1-MCP application (M)							
No 1-MCP	5.9	28.6	12.2	16.4	17.4	16.5	13.4
1-MCP	5.5	29.3	12.7	16.6	16.5	16.1	12.8
Water regime (W)							
Water-stressed	5.7	29.1	10.1	8.9	8.8	12.0	6.5
Well-watered	5.7	28.8	14.7	24.1	25.1	20.6	19.6
ANOVA				*P > F*			
M	0.0801	0.8244	0.7995	0.8733	0.5095	0.6507	0.6716
W	0.8075	0.9215	0.0113	<.0001	<.0001	<.0001	<.0001
M x W	0.6631	0.8769	0.7536	0.7737	0.441	0.536	0.9398
	Stomatal conductance (mol H_2O m^{-2} s^{-1})						
1-MCP application (M)							
No 1-MCP	0.53	1.00	0.30	0.65	0.59	0.52	0.43
1-MCP	0.36	0.98	0.30	0.60	0.56	0.48	0.40
Water regime (W)							
Water-stressed	0.42	1.03	0.18	0.10	0.08	0.19	0.05
Well-watered	0.47	0.95	0.42	1.15	1.07	0.81	0.78
ANOVA				*P > F*			
M	<.0001	0.8711	0.9269	0.5537	0.6923	0.577	0.8112
W	0.2758	0.5597	<.0001	<.0001	<.0001	<.0001	<.0001
M x W	0.0094	0.9561	0.9645	0.7094	0.8346	0.7578	0.9086

Table III.1 continued.

Source	Days after 1-MCP treatment						
	1	3	5	7	9	11	13
	Intercellular CO_2 (μmol CO_2 mol air^{-1})						
1-MCP application (M)							
No 1-MCP	326	258	102	234	200	249	190
1-MCP	316	254	166	221	180	236	180
Water regime (W)							
Water-stressed	322	256	73	177	110	209	107
Well-watered	319	256	266	278	270	276	263
ANOVA				*P > F*			
M	0.0012	0.3757	0.4564	0.3724	0.4443	0.2992	0.5037
W	0.3489	0.9688	0.0040	<.0001	<.0001	<.0001	<.0001
M x W	0.0108	0.5809	0.4839	0.5599	0.5531	0.2958	0.6556

1-MCP treatment on carbon net assimilation, except at 1 DAT when plants treated with 1-MCP showed better carbon assimilation than untreated plants (Table III.1).

Significant differences in stomatal conductance (g_s) in response to water regime started at 5 DAT and lasted throughout the entire study (Table III.1 and Fig. III.3 B). At this same time, well-watered plants had ψ_{wl} around -1.0 MPa compared to a value of -1.8 MPa for stressed plants. At 7 DAT, water stress decreased stomatal conductance by 93% when compared to well-watered conditions (Table III.1 and Fig. III.3 B). Soil water content differences were evident by 3 DAT, while ψ_{wl} showed no differences until 5 DAT (Fig. III.1). Thus, cotton plants may have been able to cope with water stress by adjusting their osmotic potential (not measured) to maintain a stable ψ_{wl} that allowed normal physiological functioning (Nepomuceno et al., 1998; Turner et al., 1986), since differences in A and g_s became obvious only when cotton plants reached a moderate level of water stress (ψ_{wl} = -1.8 MPa) at 5 DAT. The g_s responded in a similar manner as A to declining ψ_{wl}, and also showed an exponential relationship with ψ_{wl} (Fig. III.3 A). It was also obvious that g_s (Fig. III.3 B) followed the PAR pattern shown in Fig. III.2 B.

Stomatal (conductance) and non-stomatal (in chloroplasts) processes can impede the assimilation of carbon dioxide by leaves. Even though both processes are synchronized, one can inhibit carbon assimilation more than the other as stress progresses (Faver et al., 1996). Faver et al. (1996) observed the importance of non-stomatal limitations in A: while g_s declined 45%, A and C_i (intercellular CO_2) was reduced by no more than 12% under water stress. In the present research, non-stomatal

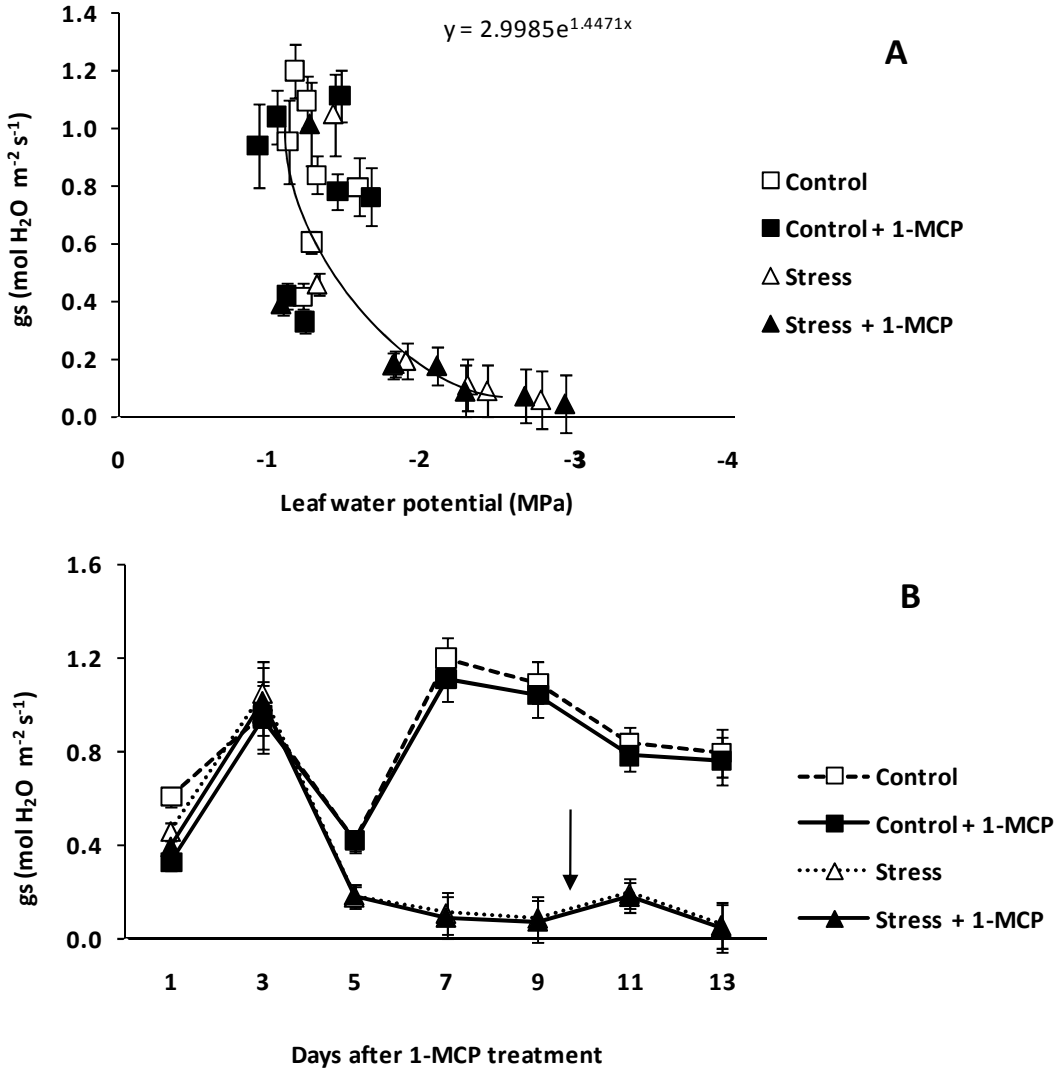

Fig. III.3. Relationship of stomatal conductance to leaf water potential (A); stomatal conductance during the experiment (B) for well-watered (control, and control plus 1-MCP) and water-stressed (stress, and stress plus 1-MCP) cotton plants in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009. The arrow denotes when watering of stressed plants was initiated with the amount of water necessary to bring pots slightly above 8% MCW. This occurred on the evening of 9 DAT and was resupplied again whenever plants reached water status below the wilting point. Plants were thus kept at a constant level of stress. Bars represent SE where greater than the symbol.

influences in A were detected when A and g_s of well-watered plants (Figs. III.2 B and 3 B) decreased nearly 52% at 5 DAT, while C_i was unaltered (Fig. III.4 A).

This 52% decrease in A and g_s may have been in response to a reduction in PAR (Fig. III.2 B) due to the overcast environment. Thus, non-stomatal factors impeded the C_i availabe to be fixed in the chloroplast as A was reduced while C_i was kept at a constant level throughout the experiment under well-watered conditions. Another possible explanation for this reduction in carbon assimilation under high intercellular CO_2 was the increase in mesophyll resistance that resulted in low A rates and high C_i levels as speculated earlier by Ephrath et al. (1990) who found similar responses in cotton grown in the field under water stress.

There was a 1-MCP application x water regime interaction for stomatal conductance at 1 DAT (Table III.1) driven by 1-MCP that showed low g_s under well-watered conditions (Fig. III.5 B). Consequently, this difference in g_s due to 1-MCP was evident when plants were grown under well-watered conditions but not under water deficit stress. Intercellular CO_2 concentration remained near constant in well-watered plants during this study, while C_i levels in water-stressed plants decreased at 5 DAT until the conclusion of evaluations (Table III.1 and Fig. III.4 A). A linear relationship was found between C_i and ψ_{wl}, with C_i decreasing together with ψ_{wl} (Fig. III.4 B). Water-stressed treatments dropped as much as 78% of their C_i due to drought effects at 5 DAT (Fig. III.4 A). Similar trends of C_i decreases in cotton plants under water stress have been reported by Ephrath et al.(1990) and Faver et al. (1996). However, under severe

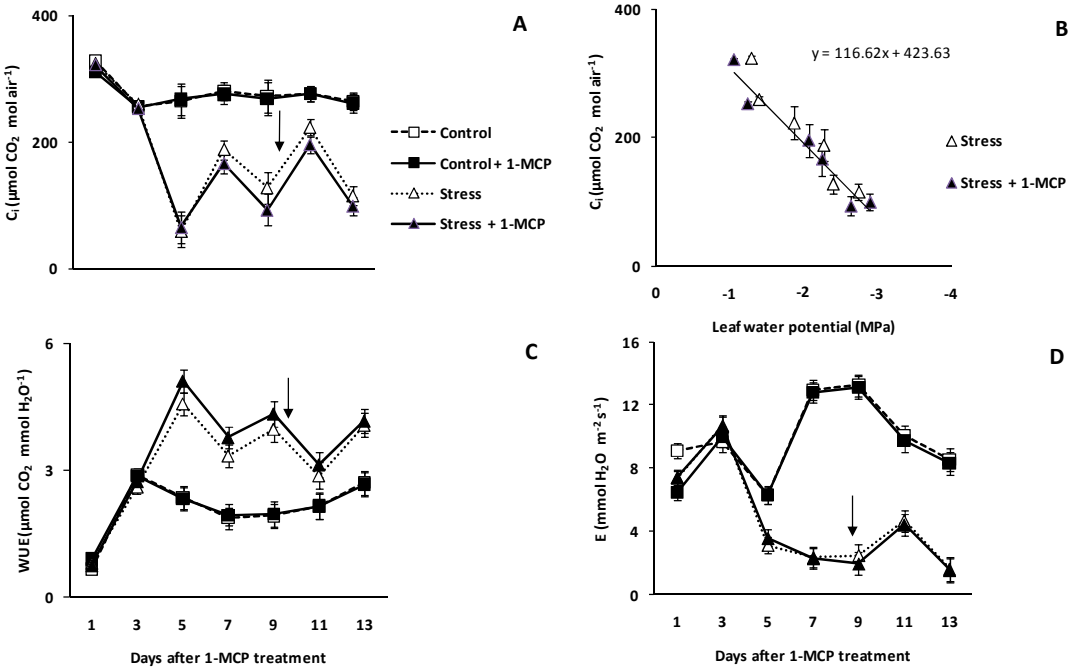

Fig. III.4. Intercellular CO_2 (A), the relationship of intercellular CO_2 to leaf water potential (B), water use efficiency (C), and transpiration rate (D) for well-watered (control, and control plus 1-MCP) and water-stressed (stress, and stress plus 1-MCP) cotton plants in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009. The arrow denotes when watering of stressed plants was initiated with the amount of water necessary to bring pots slightly above 8% MCW. This occurred on the evening of 9 DAT and was resupplied again whenever plants reached water status below the wilting point. Plants were thus kept at a constant level of stress. Bars represent SE where greater than the symbol.

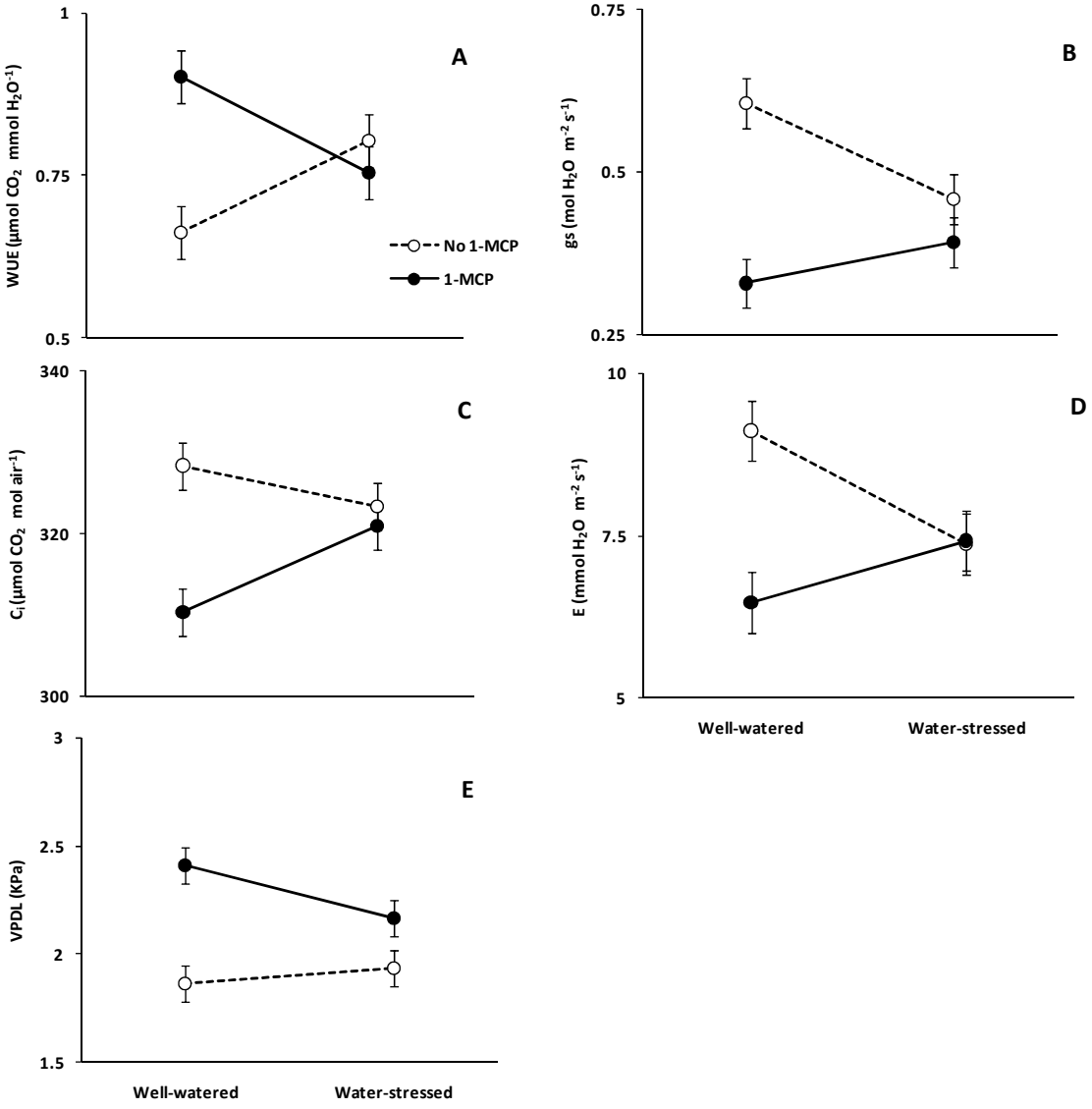

Fig. III.5. 1-MCP application by water regime interactions for water use efficiency (A), stomatal conductance (B), intercellular CO_2 (C), transpiration rate (D), and leaf vapor pressure deficit (E) 1 d after 1-MCP treatments were initiated of cotton plants in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009. Bars represent SE where greater than the symbol.

water stress Ephrath et al.(1990) observed an increase in C_i in cotton when g_s was low and concluded that mesophyll resistance (non-stomatal process) was the main limiting factor for photosynthetic processes under severe water stress instead of the C_i availability. These findings are not supported by the present work or that of Faver et al. (1996). Faver et al. (1996) reported that A and C_i reductions paralleled g_s as water stress intensified (ψ_{wl} lower than -1.5 MPa), concluding that even though non-stomatal factors may have contributed with a decrease in A and C_i, stomatal resistance was the main cause of such a decrease. The findings of Faver et al. (1996) agreed with the current study in which A (Fig. III.2 B) was nearly identical to g_s (Fig. III.3 B) curves under either moderate or severe water deficit stress. It was speculated that one of the reasons why in severe drought stress studies Ephrath et al. (1990) observed high C_i values under low g_s was that stomata took much longer to adjust to the enclosed chamber of the instrument. If insufficient time was given for stomatal adjustment prior to the data point collection, it could have led to a increase in C_i, not reflecting what occurs in reality. The current portable photosynthesis system from LI-COR (LI-COR Inc., Lincoln, NE) used in the present research provides the option to monitor curves as A and g_s, thus allowing the investigator to record data points after stability is reached. The Li-Cor 6000 used by Ephrath et al.(1990) did not provide such a resource. Therefore, knowing when to record a data point was more of a challenge, and if data point were recorded prior to stomatal adjustment, C_i values had a greater chance of being incorrect. An interaction for 1-MCP application x water regime was observed for intercellular CO_2 concentration at 1 DAT (Table III.1) caused by 1-MCP inducing a lower C_i under well-watered conditions

Table III.2. Water use efficiency, transpiration rate, leaf vapor pressure deficit, and leaf temperature from 1 to 13 days after 1-MCP treatments were initiated of cotton plants in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009.

	Days after 1-MCP treatment						
Source	1	3	5	7	9	11	13
	Water use efficiency (μmol CO_2 mmol H_2O^{-1})						
1-MCP application (M)							
No 1-MCP	0.73	2.8	4.7	2.6	3.0	2.5	3.4
1-MCP	0.83	2.8	3.7	2.9	3.2	2.6	3.4
Water regime (W)							
Water-stressed	0.78	2.7	6.1	3.6	4.2	3.0	4.1
Well-watered	0.78	2.9	2.3	1.9	2.0	2.2	2.7
ANOVA				*P > F*			
M	0.0250	0.7433	0.4511	0.334	0.4923	0.691	0.9215
W	0.9474	0.2021	0.0064	<.0001	<.0001	0.0084	<.0001
M x W	0.0011	0.6147	0.4672	0.4915	0.5927	0.6636	0.8158
	Transpiration rate (mmol H_2O m^{-2} s^{-1})						
1-MCP application (M)							
No 1-MCP	8.2	10.2	4.7	7.7	7.9	7.4	5.1
1-MCP	6.9	10.3	4.9	7.5	7.5	7.1	4.9
Water regime (W)							
Water-stressed	7.4	10.7	3.4	2.3	2.2	4.6	1.6
Well-watered	7.8	9.8	6.3	12.9	13.2	9.9	8.4
ANOVA				*P > F*			
M	0.0083	0.8481	0.665	0.8652	0.6398	0.667	0.8425
W	0.3947	0.2003	<.0001	<.0001	<.0001	<.0001	<.0001
M x W	0.0062	0.8118	0.6913	0.9316	0.7896	0.9461	0.9284

Table III.2 continued.

	Days after 1-MCP treatment						
Source	1	3	5	7	9	11	13
	Leaf vapor pressure deficit (KPa)						
1-MCP application (M)							
No 1-MCP	1.9	1.6	2.3	2.3	2.5	2.1	2.5
1-MCP	2.3	1.6	2.2	2.3	2.6	2.3	2.7
Water regime (W)							
Water-stressed	2.1	1.6	2.7	2.9	3.3	2.7	3.6
Well-watered	2.1	1.5	1.8	1.6	1.8	1.7	1.6
ANOVA				*P > F*			
M	<.0001	0.6839	0.7428	0.8596	0.5531	0.2092	0.4464
W	0.3087	0.3309	0.0002	<.0001	<.0001	<.0001	<.0001
M x W	0.0684	0.9944	0.9527	0.8153	0.8421	0.3207	0.8722
	Leaf temperature (□C)						
1-MCP application (M)							
No 1-MCP	28.5	30.4	31.7	32.2	33.3	30.8	31.7
1-MCP	29.5	30.6	31.6	32.4	33.5	31.1	31.8
Water regime (W)							
Water-stressed	29.0	30.9	32.6	33.3	35.0	32.0	34.0
Well-watered	28.9	30.2	30.7	31.3	31.8	29.9	29.6
ANOVA				*P > F*			
M	<.0001	0.6823	0.893	0.7513	0.779	0.5084	0.882
W	0.6707	0.1317	0.0686	<.0001	<.0001	0.0002	<.0001
M x W	0.9629	0.8664	0.9093	0.6083	0.9988	0.3989	0.8088

(Fig. III.5 B). Thus, differences in C_i values due to 1-MCP at 1 DAT were evident when plants were well-watered but not when under water deficit stress.

Water use efficiency (WUE) of cotton plants on a leaf area basis was calculated as A/E, with E being transpiration rate. The WUE of cotton plants under water deficit stress was improved relative to well-watered plants when stressed plants were under moderate water stress (ψ_{wl} = -1.8 MPa) at 5 DAT. This increased improvement in WUE continued until evaluations were ceased (Table III.2 and Fig. III.4 C). Pettigrew (2004b) also observed improved WUE when cotton was grown under dryland conditions. In the current study, WUE was the mirror image of C_i (Fig. III.4 A and C), because as moisture deficit progressed, g_s was reduced (Fig. III.3), thus decreasing C_i. Therefore, at the same time that stressed plants had less CO_2 available to be fixed, they also were losing less water. This was confirmed by less transpiration (E; Table III.2 and Fig. III.4 D) and the increased leaf-to-air vapor pressure deficit (VPDL; Table III.2 and Fig. III.6 A). The photosynthetic apparatus is not affected until severe drought stress is reached (Karukstis, 1991; Starman and Lombardini, 2006). Subsequently, stressed plants were more efficient in assimilating the available carbon (Ci) per unit of water vapor lost (E). However, one of the effects of having less water available for transpiration is the increase in leaf temperature (Fig. III.6 B and Table III.2). Stressed plants showed a significant increase in temperature that was initiated at 5 DAT (ψ_{wl} = -1.8 MPa) until the end of the evaluations. High temperatures can be detrimental to the integrity of the photosynthetic apparatus (Sharkey, 2005), consequently affecting plant growth and development.

There were 1-MCP application x water regime interactions for water use efficiency, transpiration rate, and leaf vapor pressure deficit observed at 1 DAT (Table III.2 and Fig. III.5). The 1-MCP treatment increased WUE and VPDL, since it decreased g_s and E under well-watered conditions. Though, 1-MCP definitely impacted physiological processes in cotton, this effect was brief and already unnoticeable on the third day following its application. Thus, is it necessary to reapply 1-MCP for its effects to persist, and if so, what would be the right time for a reapplication? Ethylene emissions of cotton plants experiencing water stress showed a shift in pattern of evaluation at 7 DAT. Both the well-watered and water-stressed plants when treated with 1-MCP changed their patterns of ethylene production after 7 DAT: plants that exceeded the ethylene synthesis of well-watered plants prior to 7 DAT became lower and those that were lower, started to be higher than the control (unpublished data). The current study data agreed with our previous findings by reinforcing the need of a 1-MCP reapplication prior to 7 d after last application.

Plant Mapping, Chlorophyll Content and Biomass Evaluations

The rate at which cotton plants grow can be estimated by the mainstem internode length, and when considered together with the number of nodes and plant height can be used to assess the treatment effects on plant growth and development (Boquet et al., 2004). Stunted growth is a very evident and self-explanatory plant response to water deficit stress (Pettigrew, 2004b). During 22 days of continuous water deficit stress, in

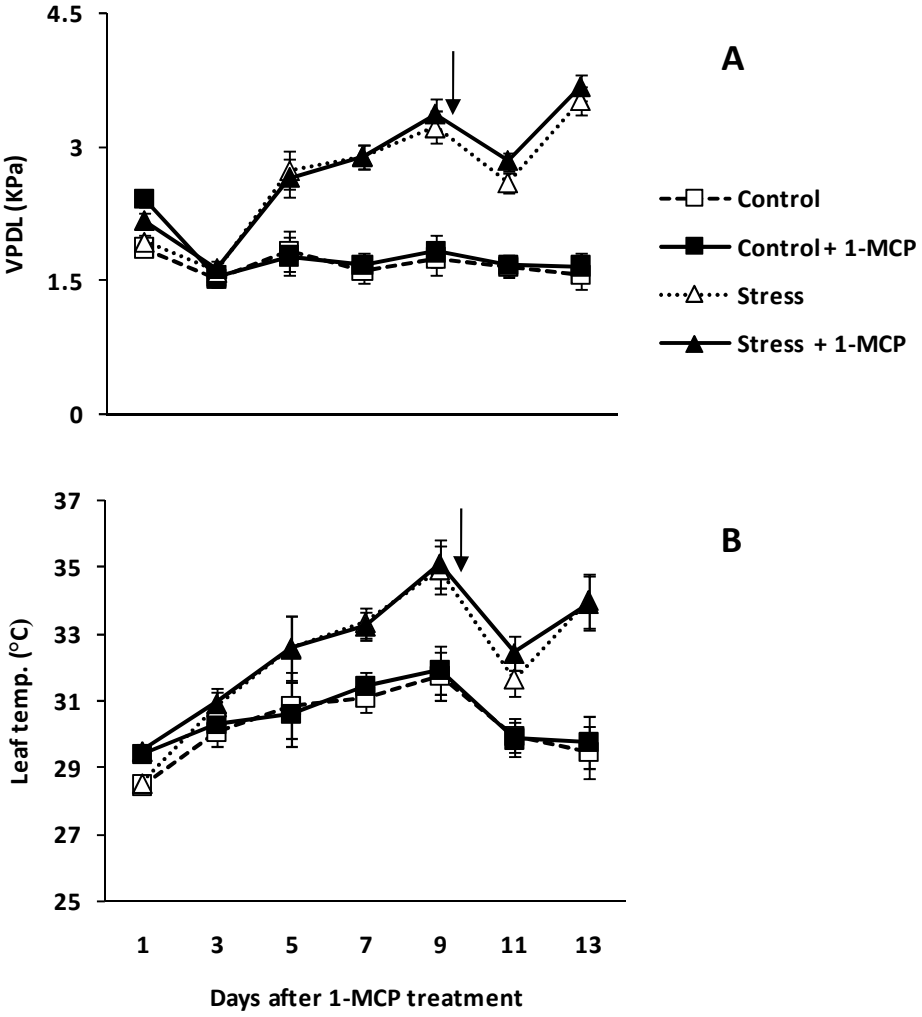

Fig. III.6. Leaf vapor pressure deficit (A), and leaf temperature (B) for well-watered (control, and control plus 1-MCP) and water-stressed (stress, and stress plus 1-MCP) cotton plants in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009. The arrow denotes when watering of stressed plants was initiated with the amount of water necessary to bring pots slightly above 8% MCW. This occurred on the evening of 9 DAT and was resupplied again whenever plants reached water status below the wilting point. Plants were thus kept at a constant level of stress. Bars represent SE where greater than the symbol.

which the last 13 days were continuously near the wilting point, drought detrimentally impacted plant height. Plant height was reduced by 33% and average internode length was reduced by 21% when compared to plants grown under well-watered conditions (Table III.3). Short plant stature is common under water deficit stress (Ball et al., 1994; Pettigrew, 2004b). Following a recovery period of 6 days after water stress had been applied for 6 days, Ball et al. (1994) reported that cotton plants exposed to water stress completely ceased height expansion, suffering a 22% reduction in height when compared with control.

The 1-MCP compound rather than water deficit stress was responsible for a reduced number of vegetative nodes along the mainstem. The number of vegetative nodes was decreased 13% (Table III.3). There was a 1-MCP application x water regime interaction for the number of reproductive nodes (Table III.3 and Fig. III.7 A). The 1-MCP treatment improved reproductive node numbers by 9 and 17% when plants were well-watered and water-stressed, respectively (Fig. III.7 A). Thus, 1-MCP contributed most to the number of reproductive nodes under water stress. Both 1-MCP application and water regime impacted the final number of nodes on the mainstem (Table III.3). Water stress decreased the mainstem node number by 16%, while 1-MCP increased this number by 3%.

Nodes above white flower (NAWF) refers to the number of mainstem nodes that are above a reproductive (sympodial) branch which has a white flower in its 1st fruiting position. NAWF assessment provides researchers the progression of the reproductive

Table III.3. Plant height, internode length, number of vegetative, reproductive and mainstem nodes, nodes above white flower (NAWF) 22 days after 1-MCP treatments were initiated of cotton plants in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009.

Source	Plant height	Internode length	Vegetative nodes	Reproductive nodes	Mainstem nodes	NAWF
	cm					
1-MCP application (M)						
No 1-MCP	87.3	4.40	7.70	11.9	19.6	4.75
1-MCP	88.8	4.33	6.73	13.6	20.3	4.75
Water regime (W)						
Water-stressed	70.7	3.85	7.13	11.1	18.2	3.75
Well-watered	105.4	4.88	7.30	14.4	21.7	5.75
ANOVA				*P > F*		
M	0.5625	0.4932	0.0006	<.0001	0.0015	1.00
W	<.0001	<.0001	0.506	<.0001	<.0001	<.0001
M x W	0.6870	0.8188	0.1586	0.0380	0.8994	0.2806

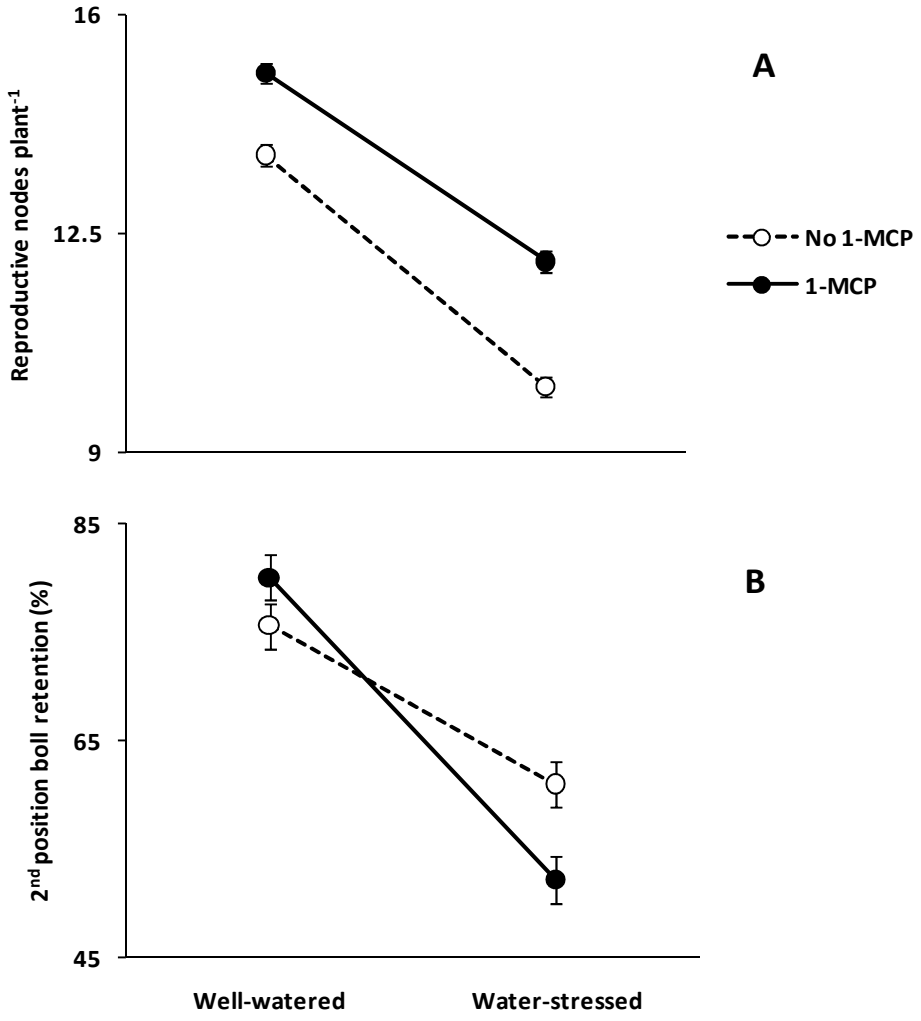

Fig. III.7. 1-MCP application by water regime interactions for the number of reproductive nodes (A), and second position boll retention (B) 22 days after 1-MCP treatments were initiated of cotton plants in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009. Bars represent SE where greater than the symbol.

development phase, and where the plant is relative to its maturity (Pettigrew, 2004a). Water regime was the only factor to impact NAWF counts in the investigation (Table III.3). Well-watered cotton plants had 35% more nodes above the white flower than those that were water-stressed. This may suggest that well-watered plants extended their vegetative phase after the initiation of their reproductive growth period, causing the plant to extend its reproductive cycle (maintaining flowering longer) and delaying crop maturity by pushing back cutout. Cutout occurs when the cotton plant starts to allocate more resources towards existing bolls, by reducing its vegetative and flowering production (Pettigrew, 2004b). Cotton has presumably reached cutout when NAWF count is at 5 (Bourland et al., 1992). The time at which NAWF was first conducted for the current study showed that water-stressed plants had reached the cutout (NAWF = 4), while the well-watered plants were still growing (NAWF = 6). Pettigrew (2004a) also reported a difference in plant development due to water deficit. He observed that irrigated plots had a 6-day delay in cutout when compared to dryland plots, which conferred a longer flowering interval to irrigated plants.

The extended growing season and delay in crop maturity caused by the well-watered regime (NAWF = 6) significantly increased the number of green bolls per plant basis. Even though there was a delay in crop maturity confirmed by a high NAWF value of well-watered plants, this delay was not reflected in the number of open bolls, since well-watered plants had 100% more open bolls than stressed plants. It was expected that stressed plants would have a higher number of open bolls, since these plants reached their maturity (NAWF = 4) earlier than plants well-watered. Thus, it was speculated that

this lack of open bolls at 22 DAT by stressed plants was a reflection of a numerically higher fruit abscission (6%) and a significantly lower whole plant boll retention (30%; Table III.4). This combination of high abscission and low whole plant retention observed was caused by the loss of young bolls formed earlier in the reproductive season, based upon the bolls located in fruiting positions 1 and 2. Water-stressed plants retained 28% less bolls in the first fruiting position (Table III.4). As for the bolls in the second fruiting position, water-stressed plants retained 35 and 19% less bolls with and without 1-MCP, respectively. This was the result of a 1-MCP application x water regime interaction for second position boll retention (Fig. III.7 B).

Fruit abscission was quantified based on the total number of scars left on branches after a reproductive structure was lost. The plant mapping data revealed that only 1-MCP affected fruit abscission even overcoming the known effect of drought in abscission (Table III.4). Drought numerically increased abscission only by 6%, when compared to the well-watered regime. In unpublished data, 1-MCP temporarily increased ethylene emissions of cotton leaves above the untreated control one day after its application. Since ethylene is one of the main triggers in abscission (Guinn, 1976; Mattoo and Suttle, 1991; Morgan et al., 1992), this early burst of ethylene could have resulted in the high fruit shed (22%) observed 22 d after 1-MCP application. Due to significant shed of fruit by 1-MCP treated plants followed by a possible increase in photoassimilate availability, these plants had a 13% increase in the number of squares (Table III.4), which may have led to the 22% higher reproductive weight (reproductive weight = combined dry weight of squares, blooms and bolls; Table III.5). Square

Table III.4. Squares, green bolls, open bolls, and abscised fruit numbers, first position, second position, as well as whole plant boll retentions of cotton plants 22 days after 1-MCP treatments were initiated in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009.

Source	Square number	Green boll number	Open boll number	Abscised fruit number	1st position boll retention	2nd position boll retention	Whole plant retention
					%		
1-MCP application (M)							
No 1-MCP	10.0	4.5	0.13	6.78	59.68	68.30	64.93
1-MCP	11.5	4.7	0.08	8.68	56.03	66.13	62.05
Water regime (W)							
Water-stressed	4.6	4.1	0.00	7.95	48.30	56.60	52.40
Well-watered	16.9	5.1	0.20	7.50	67.40	77.83	74.58
ANOVA				*P > F*			
M	0.0098	0.4707	0.3103	<.0001	0.2200	0.3113	0.1854
W	<.0001	0.0192	0.0002	0.2381	<.0001	<.0001	<.0001
M x W	0.6260	0.2673	0.3103	0.0702	0.6471	0.0035	0.5076

Table III.5. Dry matter partitioning and chlorophyll (Chl) content of cotton plants 22 days after 1-MCP treatments were initiated in the Borlaug Center greenhouses, at Texas A&M University, 2008-2009.

Source	Leaf area	Chl content	Specific leaf weight	Vegetative weight	Reproductive weight	Harvest Index†
	$cm^2\ plant^{-1}$		$g\ m^{-2}$	$g\ plant^{-1}$		
1-MCP application (M)						
No 1-MCP	1119	44.1	37.0	6.70	2.65	0.205
1-MCP	1297	45.1	37.3	7.58	3.38	0.227
Water regime (W)						
Water-stressed	772	47.5	41.2	4.79	2.76	0.256
Well-watered	1644	41.7	33.1	9.48	3.28	0.177
ANOVA				*P > F*		
M	0.052	0.4344	0.8445	0.0164	0.0813	0.3439
W	<.0001	<.0001	<.0001	<.0001	0.2114	0.0018
M x W	0.7071	0.9947	0.751	0.4989	0.8910	0.8784

† Harvest index = reproductive dry weight/total aboveground dry weight.

number was also decreased by water regime (Table III.4). Drought decreased square number by 72%.

Dry matter partitioning was impacted by water regime (Table III.5). Water-stressed plants had a slow leaf growth rate and consequently a reduced leaf size (unpublished data). Stressed plants were 33% shorter (Table III.3), produced 40% less total leaf weight (data not shown), and 53% less total leaf area, which when divided by the total leaf weight provided the specific leaf weight (SLW). Drought increased SLW by 20%. Pettigrew (2004b) reported that plants grown under dryland production had a 12% increase in SLW, and he speculated that these leaves may have been denser or thicker than leaves of irrigated plants. Chlorophyll (Chl) content was only affected by water regime (Table III.5). Water-stressed plants had 12% more chlorophyll than well-watered plants. At least two possibilities exist for why there was higher Chl content in stressed plants: i) drought-stressed plants had smaller and thicker leaves, causing higher Chl readings; ii) SPAD meters are used to estimate Nitrogen (N) fertilization based on a control-check (known N rate); since fertilization application was suspended in this study to the end of evaluations, this may have caused a ‘dilution’ effect. Well-watered plants may have translocated N resources to new growing areas due to the fact that irrigated plants had an extended growing season (NAWF = 6) resulting in lower chlorophyll readings. The SLW data revealed stressed plants had thicker/denser leaves which may have led to more Chl per leaf basis and consequently more N as well, since every Chl molecule carries four N atoms.

Vegetative weight, which consisted of the dry weight of stems and petioles, was impacted by water regime (Table III.5). Drought decreased the vegetative weight of cotton plants by 50%, but did not impact reproductive weight. Thus, this combination of reduced vegetative weight without reducing reproductive weight may have caused a 31% higher harvest index in cotton plants under drought conditions when compared to well-watered plants. As a result of higher WUE in water-stressed plants (previously discussed; Table III.2 and Fig. III.4 C), harvest index was also increased. Increased WUE and harvest index of stressed plants reported in this study are in agreement with previous research (Pettigrew, 2004b) in which WUE was higher and harvest index increased by 30% for dryland cotton. Gerik et al.(1996) noted the importance of a high harvest index towards lint yield. They observed that a higher harvest index (partitioning of more dry matter to bolls) was one of the major contributors to increased lint yield in one of the varieties in their study.

Considering that vegetative nodes contribute little to overall lint yield, and that the vast majority of the cotton lint yield comes from the reproductive nodes (originate on sympodial branches), 1-MCP showed a significant potential to improve lint yield (not evaluated) in cotton. It not only reduced the number of vegetative nodes, but also increased reproductive nodes per plant basis by 17%. This occurred mainly when cotton plants were exposed to water stress deficit for 22 d (Fig. III.7 A) during the reproductive phase. Thus, 1-MCP apparently allowed plants to overcome stress and set more reproductive nodes. Boquet et al. (2004) stated that mainstem node number is important to lint yield because nodes produce branches that can support 1 to 3 bolls. They reported

that an increase in cotton mainstem nodes produced higher lint yield. However, this greater number of reproductive nodes caused by 1-MCP did not improve harvest index. The 1-MCP caused an increase in vegetative weight by 12%, but it also caused a 22% increase in reproductive weight (as a consequence of a 22% abscission increase, discussed earlier). Since harvest index was calculated by dividing reproductive dry weight by total aboveground dry weight, these large increases in vegetative and reproductive weight due to the 1-MCP may explain the lack of response of the harvest index compared to water stress which had the best harvest index.

CONCLUSIONS

Gas Exchange Evaluations

In conclusion, gas exchange analysis revealed that water deficit stress significantly impacted cotton plants at moderate water stress level (ψ_{wl} = -1.8 MPa) 5 days after 1-MCP application. Water stress decreased stomatal conductance, intercellular CO_2, transpiration rate, and CO_2 net assimilation rate; and increased leaf vapor pressure deficit, leaf temperature, as well as water use efficiency. The 1-MCP treatment briefly affected gas exchange parameters by increasing CO_2 net assimilation rate at 1 DAT, as it caused well-watered plants to be more efficient in assimilating more carbon dioxide per unit of water. At 1 day after 1-MCP application in well-watered conditions as a result of interaction, the 1-MCP decreased stomatal conductance, intercellular CO_2, transpiration rate, and increased vapor pressure deficit, and water use efficiency. However, when water stress started to impact cotton plants at 5 DAT, 1-MCP did not ameliorate any of the adverse effects of water stress on gas exchange parameters.

Plant Mapping, Chlorophyll Content and Biomass Evaluations

Plant mapping, dry matter partitioning and chlorophyll content data showed that water deficit stress reduced plant height, internode length, nodes above white flower, total leaf area and weight, vegetative weight, number of squares, reproductive growth, number and retention of bolls. On the other hand, drought increased specific leaf weight, chlorophyll content, and harvest index.

1-MCP treatments had little or no positive effect on plant mapping, dry matter partitioning and chlorophyll content. The application of 1-MCP decreased the number of vegetative nodes, and increased the number of squares and reproductive nodes by 9% when plants were well-watered and by 17%, when under stress. The 1-MCP treatment showed a potential to improve lint yield in cotton, as it increased reproductive nodes per plant basis mainly for cotton under water stress during its reproductive phase. However, this greater number of reproductive nodes did not lead to a better harvest index, since 1-MCP caused high fruit abscission. In unpublished data, it was observed that 1-MCP temporarily increased ethylene emission in cotton leaves above the untreated control one day after its application. Because ethylene is one of the main stimuli in abscission, it was speculated that this burst of ethylene early in the reproductive stage was one of the major factors for the high fruit shed that was observed 22 days after 1-MCP application.

CHAPTER IV

ABIOTIC STRESS EFFECTS ON PLANT GROWTH AND YIELD COMPONENTS OF 1-MCP TREATED COTTON PLANTS

OVERVIEW

Boll abortion is increased when cotton (*Gossypium hirsutum* L.) experiences various stresses during its reproductive development that can consequently reduce lint yield. Prior to abscission, a burst in ethylene is observed which may be assumed to be the signal necessary to initiate abscission of that particular structure. It is desirable to prevent fruit loss that may be induced by the peak in ethylene prior to abscission. One potential option to cope with the loss of cotton reproductive structures is the use of ethylene inhibitors. Thus, the objective of this investigation was to determine the impact of 1-methylcyclopropene (1-MCP) on growth and yield components of cotton plants treated with ethephon (ethylene synthetic hormone) under field conditions. Field studies were conducted as a randomized complete block design with four replications in 2007and 2008. Treatments were three rates of 1-MCP in combination with a surfactant applied at mid-bloom. One day later, ethephon was applied as a source of abiotic stress. At harvest, the fruit set in the upper portion of the canopy was influenced by 1-MCP. It had a greater number of full size, yet immature bolls, which potentially could have had a positive influence in the lint yield. However, ethephon caused the highest lint yield since ethephon treated plants had more open as well as total bolls in the lower canopy at harvest. In conclusion, 1-MCP did improve growth and yield components mainly in the

upper portion of plants canopy at harvest, but such an improvement was not converted into lint yield.

INTRODUCTION

Based on research conducted in the U.S. (Boquet et al., 2004; Worley et al., 1974; Wu et al., 2005), the variable which contributed the most to lint yield was the number of bolls area^{-1}. However, boll abortion is increased when cotton plants are under severe stress during their reproductive development that consequently reduces lint yield (Gerik et al., 1996; Pettigrew, 2004a; Turner et al., 1986). Morgan et al. (1992) observed that there was a burst in ethylene levels in cotton leaves that lasted 4 days prior to occurrence of abscission. The authors suggested that this peak in ethylene may be the signal necessary to initiate cell wall hydrolysis in the abscission zone followed by abscission of that particular structure.

It is desirable to protect yield by preventing fruit loss induced by the peak in ethylene prior to abscission. Because the number of bolls area^{-1} contributes the most to cotton lint yield (Boquet et al., 2004; Worley et al., 1974; Wu et al., 2005), and the assumed role ethylene plays in inducing abscission (Guinn, 1976; Steel and Torrie, 1980), it is necessary to look for alternatives that could reduce or prevent abortion of cotton bolls under stress. Preventing loss of flowers and young fruit is essential in cotton yield enhancement (Heitholt et al., 1993); thus, ethylene inhibitors could provide an alternative for coping with the loss of reproductive structures, in an effort to improve cotton yield.

The compound 1-methylcyclopropene (1-MCP) is a gaseous ethylene antagonist that blocks ethylene receptors, consequently inhibiting its perception and preventing ethylene effects in the plant tissues (Blankenship and Dole, 2003; Sisler and Serek, 1997). The affinity of 1-MCP to ethylene receptors is 10x greater than the affinity of ethylene to its receptors (Blankenship and Dole, 2003). 1-MCP is widely used in horticultural production (Fan and Mattheis, 2000). Studies in horticulture mainly focused on post-harvest physiology of climacteric fruit to counter the detrimental effects of ethylene. These studies showed that the compound impacts a variety of physiological processes, such as decreasing ethylene synthesis (Blankenship and Dole, 2003; Dong et al., 2001; Jeong et al., 2002), respiration (Blankenship and Dole, 2003; Dong et al., 2001; Fan and Mattheis, 2000), and chlorophyll degradation (Blankenship and Dole, 2003; Fan and Mattheis, 2000; Jiang et al., 2002), thus extending shelf-life (Fan and Mattheis, 2000).

The objectives of this study were to determine the impact of 1-MCP on growth and yield components of cotton plants treated with ethephon as a source of abiotic stress under field conditions. Even though cotton plants have the ability to compensate for early season fruit loss (Stewart et al., 2001), as a secondary objective, it was also important to know to what extent plants can compensate for fruit loss during the late season.

MATERIALS AND METHODS

Cultural Practices

Studies were conducted at the Texas AgriLIFE Field Laboratory in Burleson County, TX, on a Weswood silt loam soil with a pH of 7.9. Cotton ('Stoneville 4554 B2RF') was seeded on 10 April in 2007 and 2008 at 12 seeds m^{-2}. Each plot consisted of four rows that were 1.02-m wide and 9.73-m in length. Furrow irrigation was used when necessary to avoid water stress. Fertility, disease prevention, weed and insect control followed the cultural practices for the Texas AgriLIFE Extension Service local recommendations. Harvest aids were applied with a four-row compressed air small plot sprayer equipped with hollow cone nozzles spaced at 51 cm that delivered 140.2 L ha^{-1}. Harvest aids were applied at ≈60% open boll in each study, and consisted of a combination of thidiazuron (*N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea; 0.056 kg a.i. ha^{-1}), tribufos (*S,S,S*-tributyl phosphorotrithioate; 0.421 kg a.i. ha^{-1}), and ethephon (2-chloroethyl phosphonic acid; 1.106 kg a.i. ha^{-1}).

Treatment Application and Experimental Design

Treatments were arranged as a randomized complete block design with four replications. They consisted of three rates of 1-MCP (0, 25 and 50 g a.i. ha^{-1}) in combination with the surfactant Dyne-Amic (Helena Holding Company, TN) at 0.37% v v^{-1} applied 93 d after planting (DAP; at mid-bloom). On the next day, ethephon (ethylene synthetic hormone; 292 mL ha^{-1}) was applied as a source of stress (Table IV.1). All treatments were applied as a foliar spray with 93 L ha^{-1} of water using a

compressed air small plot sprayer. The 1-MCP formulation was a soluble powder (3.8 % a.i.), which was released in contact with water.

Each experimental unit consisted of two plots side-by-side, called paired-plots, to avoid mechanical damage to plants in the plots that were to be harvested mechanically after the crop was terminated. While one of the plots was utilized for harvest by a small plot picker (the two inside rows) the other was used to assess the crop growth and development by collecting data and plant materials during the growing season.

Since 1-MCP is volatile, there was a concern of cross-contamination among treatments. To avoid treatment contamination, paired-plots were physically isolated from others by an untreated plot (filler) in between the paired-plots within blocks. Blocks were isolated from each other by a 4-m wide alley. Zero-control paired-plots (0 g a.i. of 1-MCP ha^{-1}; 0% of surfactant v v^{-1}; and 0 mL of ethephon ha^{-1}) were isolated further by placing additional fillers across the alley ways in the blocks before and after the zero-control twin-plots.

Data Collection

Late Season Measurements

Plant height (from cotyledons to top node), counts of mainstem, vegetative, and reproductive nodes, nodes above white flower (NAWF), numbers of open bolls, green bolls, squares (flower buds), abscised fruit, and whole plant retention fruit at 1^{st} and 2^{nd} boll positions were determined 50 days after treatments (DAT) were sprayed. Data was taken from 10 randomly chosen plants from the two center rows of each designated plot. Internode length was determined by dividing plant height by mainstem node number.

Fruit abscission was calculated by counting scars left on sympodial branches at any fruiting position. NAWF counts were determined by counting the number of mainstem nodes above a reproductive (sympodial) branch with a white bloom at first fruiting position (Pettigrew, 2004a).

At Harvest Measurements

After treatments were sprayed, the top node with a visible leaf of 30 randomly chosen plants (from the two center rows per designated plot for sampling) was tagged to denote the node separating the upper (≥ node 16) and lower (≤ node 15) canopy. This separation was used to investigate if the treatments would cause growth/developmental changes on the newer portion of the plants established after spraying (designated as upper canopy), or on the pre-established portion of the canopies (lower canopy), or in both sections of plants. Immediately before machine harvest, 20 tagged plants per designated plot were sampled to determine upper and lower canopy length, total nodes, full size yet immature bolls (immature fruit), open fruit, and total fruit per plant. The two inside rows of each designated plot for machine harvest were picked to determine lint yield in once-over harvest. To avoid incorrect yield measurements due to the end-of-row effects (Holman and Bednarz, 2001) in 2008 ≈1 m of row was removed from each end of all plots prior to harvest.

Data Analysis

Data were subjected to analysis of variance using PROC MIXED (Littell et al., 2006) of SAS version 9.2 (SAS Institute Inc., 2008). Homogeneity of variance across

years was tested for each variable. Multiple mean comparisons were made using Tukey-Kramer test at $P \leq 0.05$ (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The combined data analyses did not show treatment interaction with years; however, it showed interactions between 1-MCP, surfactant, and ethephon. Thus, data were presented among treatments across years.

Late Season Measurements

Plant growth and development were affected by treatments at 50 DAT (Table IV.1). Ethephon alone and 1-MCP (25 g a.i. ha^{-1}) plus surfactant treatments had the tallest cotton plants, while ethephon plus surfactant influenced plant height the least. However, these treatments were not statistically different than the other treatments including the control. Kennedy et al. (1991) investigated the effects of ethephon on productivity of cotton plants and reported that ethephon treated plants showed height either similar to or lower than the control, suggesting a deleterious impact of ethephon on newer plant growth. The rate at which cotton plants grow can be indicated by the mainstem internode length, and together with number of nodes and plant height can be used to assess the effects of treatments on plant growth and development (Boquet et al., 2004). Treatments showed no effect on either internode length or the number of vegetative nodes (Table IV.1). Cotton plants generate nodes that produce branches which can support 1 to 3 bolls. Although the number of vegetative nodes was not affected by any treatment, ethephon alone reduced the number of reproductive nodes (Table IV.1), while all treatments with 1-MCP (independent of surfactant) were not

different than the control. This indicates that 1-MCP overcame the detrimental effect of ethephon on reproductive node numbers. However, the ethephon impact on reproductive node numbers was not enough to show any significant difference in the total number of nodes in the mainstem. Perhaps this was due to the similarities in terms of vegetative nodes and internode length. Unpublished data from associated studies with 1-MCP revealed that 1-MCP partially overcame the detrimental effects of water stress by increasing the number of reproductive nodes by 17% when compared with untreated plants also under stress.

Nodes above white flower (NAWF) counts refer to the number of mainstem nodes above a reproductive (sympodial) branch which has a white flower in the first fruiting position. The NAWF assessment provides researchers with information about where the cotton plant is in its reproductive development phase and its maturity (Pettigrew, 2004a). Both rates of 1-MCP applied without surfactant showed 100% less nodes above white flower than all the other treatments (Table IV.1), revealing a detrimental effect of 1-MCP on NAWF in the absence of the surfactant resulting in an acceleration of crop maturity. On the other hand, when both rates of 1-MCP were applied together with the surfactant, this combination ameliorated the negative effect of both 1-MCP rates alone on NAWF and preserved the normal rhythm of the crop maturity since both rates of 1-MCP plus surfactant were not different than control. This negative impact of 1-MCP on NAWF suggested that the reproductive phase of these treated plants was shortened (finishing flowering earlier) which was reflected in earlier

Table IV.1. Effects of 1-MCP, surfactant and ethephon on plant height, internode length, counts of vegetative, reproductive, and mainstem nodes, and nodes above white flower (NAWF) per plant 50 days after treatments were initiated at the Texas AgriLIFE Field Laboratory in Burleson County, TX, 2007-2008.

1-MCP	Surfactant 0.37% v v^{-1}	Ethephon 292 mL ha^{-1}	Plant height	Internode length	Vegetative nodes	Reproductive nodes	Mainstem nodes	NAWF
g a.i. ha^{-1}			cm					
0.0	-	-	81.4ab	3.2a	5.8a	15.5a	21.3a	1.1a
0.0	-	+	84.7a	3.3a	5.2a	13.9b	21.6a	1.1a
0.0	+	+	80.3b	3.3a	5.7a	14.9ab	20.6a	0.8a
25.0	-	+	82.0ab	3.4a	5.6a	14.6ab	20.2a	0.0b
25.0	+	+	86.8a	3.5a	5.5a	15.8a	21.3a	1.3a
50.0	-	+	82.0ab	3.3a	5.6a	15.2a	20.8a	0.0b
50.0	+	+	81.3ab	3.3a	5.7a	15.1a	20.8a	1.3a

cutout and consequently crop maturity. Cutout occurs when cotton plant starts to allocate more resources towards existing bolls, by reducing its vegetative and reproductive (flowering) growth (Pettigrew, 2004b). This 1-MCP detrimental stress impact on crop maturity may be comparable to that from the stress caused by drought.

Pettigrew (2004a) reported that time to cutout in drought stressed plants was shortened by 6 days resulting in a reduced flowering period compared to well-watered plants. This agrees with our unpublished data while investigating drought effects on crop maturity in greenhouse.

Decreased number of squares (flower buds) indicates that cotton plants are nearing completion of the flowering phase. Thus, in addition to low NAWF values, the shortening of flowering caused by both rates of 1-MCP plus ethephon in the absence of surfactant was also supported by a low number of square counts (Table IV.2). The loss of squares may allow cotton plants to compensate for this loss by providing more carbon allocation towards boll set and increasing boll weight (Stewart et al., 2001), and taller plants (Kennedy et al., 1991).

Kennedy et al. (1991) investigated the effects of ethephon on productivity of cotton plants by early season square removal (≈46 DAP) and reported that plant height was increased after squares were removed manually. However, no gains in plant height were observed when squares were removed by ethephon. This reported lack of height gain following ethephon square removal is in agreement with this current study. Even though ethephon caused the greatest square abscission, it did not increase plant height at 50 DAT. This may be due to a moderate level of square abscission caused by ethephon,

Table IV.2. Effects of 1-MCP, surfactant and ethephon on lint yield at harvest, numbers of green bolls, squares, and abscised fruit, and retention of whole plant, first and second fruiting position bolls per plant 50 days after treatments were initiated at the Texas AgriLIFE Field Laboratory in Burleson County, TX, 2007-2008.

1-MCP	Surfactant 0.37% v v^{-1}	Ethephon 292 mL ha^{-1}	Lint yield	Green boll number	Square number	Abscised fruit number	1st position boll retention	2nd position boll retention	Whole plant retention
g a.i. ha^{-1}			Kg ha^{-1}				%		
0.0	-	-	1348ab	7.7abc	0.34ab	21.6ab	28.9a	16.3bc	24.3a
0.0	-	+	1440a	9.4a	0.41ab	26.2a	25.8ab	19.7a	24.4a
0.0	+	+	1359ab	6.0d	0.60ab	19.6b	25.5ab	17.8ab	22.5ab
25.0	-	+	1170c	6.8bcd	0.09b	21.8ab	23.8b	18.7ab	21.4b
25.0	+	+	1208bc	8.2ab	0.84a	23.4ab	26.6ab	16.5bc	24.8a
50.0	-	+	1083c	6.2cd	0.19b	21.3ab	24.6ab	14.6c	20.7b
50.0	+	+	1207bc	6.3cd	0.54ab	20.9b	29.1a	14.6c	22.7ab

since Stewart et al. (2001) reported that only plants that had squares manually removed (100% removal) overcompensated for the loss, while plants that had squares removed by ethephon lacked compensation. They speculated that the chemical removal of squares may only have caused moderate levels of square removal, and consequently was insufficient to impact compensation following square shedding during prebloom.

Lint yield at harvest was only affected by 1-MCP (Table IV.2). Treatments containing 1-MCP had the lowest lint yields. Both rates of 1-MCP when applied with surfactant had lint yields similar to the control; however, when sprayed without the surfactant, lint yields were significantly lower than the control. Therefore, considering that ethephon in combination with the surfactant had lint yield identical to the untreated control, 1-MCP had a detrimental effect on lint yield. Treatments had no effect on fiber quality (data not shown).

Plants treated with ethephon alone showed the greatest fruit abscission counts, while the highest 1-MCP rate combined with the surfactant, as well as ethephon plus surfactant, caused the lowest fruit abscission (Table IV.2). Results indicated that the treatment that caused the highest fruit abscission in cotton, ethephon alone, was also the one that had the highest lint yield. Even though cotton plants have the ability to compensate for early season fruit loss (Stewart et al., 2001), it is important to know to what extent plants can compensate for fruit loss during the later stages of fruit development. Thus, the ethephon-stressed cotton plants were still able to compensate for the fruit loss during mid-bloom by having the highest lint yield. These findings corroborated those of Stewart et al.(2001) who reported an occurrence of

overcompensation (yield increase) in the treatment where all the squares were removed one week after squaring was initiated. In current studies, it was also possible to register this overcompensatory effect to fruit shed on cotton plants in the number of green bolls present at 50 DAT (Table IV.2 and Fig. IV.1). The highest fruit abscission caused by ethephon alone observed at 50 DAT, led to overcompensation with cotton plants setting the greatest number of green bolls.

Fruiting positions 1 and 2 are the ones that contribute the most to cotton lint yield. The highest rate of 1-MCP plus surfactant had a similar retention of bolls in the first fruiting position as the control, while the lowest rate of 1-MCP alone had the lowest retention (Table IV.2). The highest 1-MCP rate had the lowest second boll retention independent of surfactant. Boll retention numbers for the whole cotton plant at 50 DAT showed that both rates of 1-MCP sprayed alone caused the lowest whole plant fruit retention values. These lower values for first and second boll retention, as well as for whole plant retentions caused by both 1-MCP rates observed at 50 DAT reflected the detrimental impact of 1-MCP on cotton growth and development for the formulation studied in both years of this study. These values were in agreement with the detrimental effects of 1-MCP on plant height and lint yield previously mentioned.

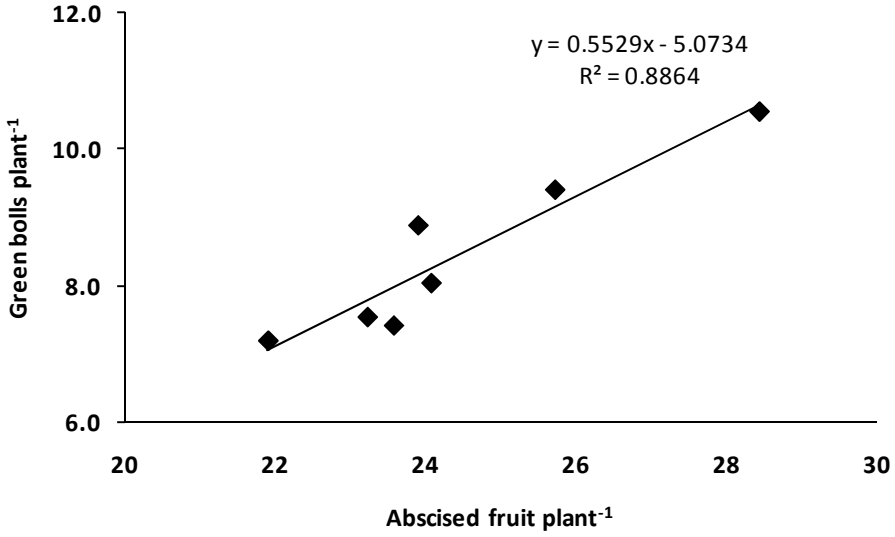

Fig. IV.1. Relationship between the number of green bolls and abscised fruit per plant at 50 days after treatments were initiated at the Texas AgriLIFE Field Laboratory in Burleson County, TX, 2007-2008.

At Harvest Measurements

The highest rate of 1-MCP positively impacted the upper portion of the plant canopy (Table IV.3). The highest rate of 1-MCP combined with the surfactant increased the upper canopy length by 29% and the number of nodes in the same canopy section by 14%, when compared to the untreated control. Boquet et al. (2004) reported cotton lint yield was associated with increases in plant height, and node number, since taller plants have more nodes that can potentially lead to more reproductive branches which have the ability to bear 1 to 3 bolls. However, such an increase in height or node number that was observed only at harvest and not at 50 DAT did not lead to increased lint yield. This upper part of the canopy had a large number of bolls (full size yet immature bolls) that were unopened (same number of open bolls as control) at harvest (Table IV.4). If this high number of full size, yet immature bolls had opened at harvest, the 50 g a.i. ha^{-1} rate of 1-MCP plus surfactant would have had a 67% higher open boll number than the control and would likely have improved lint yield.

The lowest 1-MCP rate plus surfactant also impacted the upper canopy development (Table IV.3). The total number of nodes in the upper canopy was increased by 14% when compared to the control. This increase in node number caused a 32% increase in the total number of fruit in the upper canopy in comparison with the control (Table IV.4). Nevertheless, this increase in the total fruit number in the upper canopy did not lead to increased lint yield since the majority of this fruit increase was due mainly to a 76% increase in the number of what appeared to be full size but still yet

Table IV.3. Effects of 1-MCP, surfactant and ethephon on upper canopy length, and total number of nodes per plant in the upper plant canopy at harvest, at the Texas AgriLIFE Field Laboratory in Burleson County, TX, 2007-2008.

1-MCP	Surfactant 0.37% v v^{-1}	Ethephon 292 mL ha^{-1}	Upper canopy length†	Upper canopy total node‡
g a.i. ha^{-1}			cm	
0.0	-	-	17.5b	7.4b
0.0	-	+	20.3ab	8.3ab
0.0	+	+	22.1ab	7.6b
25.0	-	+	20.8ab	8.2ab
25.0	+	+	21.6ab	8.6a
50.0	-	+	21.9ab	7.6b
50.0	+	+	24.7a	8.6a

† Canopy length above node 15.

‡ Total number of nodes above node 15.

Table IV.4. Effects of 1-MCP, surfactant and ethephon on immature fruit, open fruit, and total fruit numbers per plant in the upper and lower plant canopy sections at harvest, at the Texas AgriLIFE Field Laboratory in Burleson County, TX, 2007-2008.

1-MCP	Surfactant 0.37% v v^{-1}	Ethephon 292 mL ha^{-1}	Immature fruit†	Open fruit‡	Total fruit
g a.i. ha^{-1}			Upper canopy§		
0.0	-	-	0.5c	2.9ab	3.4b
0.0	-	+	1.4b	3.1a	4.5ab
0.0	+	+	1.2b	2.5b	3.7b
25.0	-	+	1.4ab	3.2a	4.6ab
25.0	+	+	2.1a	2.9ab	5.0a
50.0	-	+	1.3b	2.4b	3.7b
50.0	+	+	1.5ab	2.8ab	4.3ab
			Lower canopy¶		
0.0	-	-	0.55b	6.3a	6.9a
0.0	-	+	0.75a	6.2a	7.0a
0.0	+	+	0.83a	5.1b	5.9b
25.0	-	+	0.60b	5.4b	6.0b
25.0	+	+	0.50b	5.5b	6.1b
50.0	-	+	0.58b	5.0b	5.6b
50.0	+	+	0.64b	5.5b	6.1b

† Total number of full size yet immature fruit after defoliation.

‡ Total number of open fruit after defoliation.

§ Portion of plant canopy above node 15 (≥ node 16).

¶ Portion of plant canopy below node 15 (≤ node 15).

immature bolls (not cracked), instead of the number of open bolls which was similar to the control. Thus, both rates of 1-MCP showed potential to increase lint yield, but this potential was not converted into lint yield because the extra bolls set did not open in time for the mechanical harvest.

Ethephon alone resulted in the greater cotton lint yield than 1-MCP treatments although it was no better than the control (Table IV.2). Ethephon parameters investigated at harvest (Tables IV.3 and IV.4) showed some similarities to 1-MCP treatments with only two main differences: ethephon caused a greater total number of fruit located in the lower portion of the canopy with the majority being already opened when compared to the 1-MCP treatments (Table IV.4).

The high number of green bolls observed throughout the plant canopy conferred by ethephon treatment at the late season evaluations (50 DAT; Table IV.2) was likely translated into the high total number of fruit located in the lower portion of the canopy at harvest. This may explain then why ethephon treatment had higher lint yield than the 1-MCP treatments, since these open bolls were machine harvested unlike the full size, yet immature bolls (not cracked) located in the upper canopy observed in 1-MCP treated plants.

CONCLUSIONS

Both rates of 1-MCP applied without surfactant showed 100% less NAWF than the other treatments, suggesting a detrimental effect of 1-MCP on NAWF in the absence of the surfactant. Crop maturity was subsequently accelerated in these treatments. On the other hand, when both rates of 1-MCP were combined with the surfactant, the negative

effect of 1-MCP being applied alone on the number of NAWF was similar to the untreated control. Current results showed that treatments containing only 1-MCP had significantly lower lint yields than the ethephon alone.

At harvest, both rates of 1-MCP showed potential to increase lint yield due to enhanced immature fruit set in the upper canopy, but this potential was not translated into lint yield because the extra bolls set did not have time to mature for the mechanical harvest.

At harvest, ethephon treatment had greater total number of fruit located in the lower portion of the canopy. Most of these bolls were already opened. Thus, cotton plants treated with ethephon were still able to compensate for the fruit loss caused during mid-bloom. Such a compensation may have led to a higher lint yield than all the 1-MCP treatments.

CHAPTER V

SUMMARY

Part of the objectives of the greenhouse studies were to establish whether drought affects ethylene biosynthesis and the expression of related genes involved in the ethylene synthesis. This evaluation was made on detached leaves from cotton plants exposed to water deficit stress during the peak reproductive phase. Additional evaluations were made to determine whether these effects could be altered by 1-MCP. The results indicated that ethylene synthesis had a linear relationship with plant ψ_w status and ethylene synthesis. Water deficit caused a continuous decrease in ethylene synthesis, and as drought progressed ethylene production reached the lowest level of 75 ρmol g^{-1} DW h^{-1} at -2.9 MPa. The 1-MCP caused a transient climacteric stage (ethylene synthesis increase) in cotton leaves at 1 DAT reflected by short-lived increases in ethylene production. The detrimental effect of drought on ethylene levels observed was partially validated by the expression of *ACS6* and *ACO2* cotton genes. 1-MCP significantly down-regulated the expression of *ETR5*, and may have altered ethylene perception 1 d after 1-MCP application. The *GDSL* cotton gene showed a potential as a drought-responsive gene. 1-MCP showed little influence on ethylene synthesis and expression of related genes.

Other objectives of the greenhouse experiments were to establish how drought affects gas exchange, plant growth/development and yield components of 1-MCP treated cotton plants during the peak of reproductive phase. A secondary objective was to determine if gas exchange, plant growth/development and yield components responses to

drought could be altered by the presence of 1-MCP treatment. Gas exchange analysis revealed that water deficit stress significantly impacted cotton plants at moderate water stress level (ψ_{wl} = -1.8 MPa). Water stress decreased stomatal conductance, intercellular CO_2, transpiration rate, and CO_2 net assimilation rate; and increased leaf vapor pressure deficit, leaf temperature, as well as water use efficiency. The 1-MCP treatment briefly affected gas exchange parameters. It caused well-watered plants to be more efficient in assimilating more carbon dioxide per unit of water only at 1 DAT. However, when water stress started to impact cotton plants at 5 DAT, 1-MCP did not ameliorate any of the adverse effects of water stress on gas exchange parameters. Plant mapping of greenhouse grown plants, dry matter partitioning and chlorophyll content data showed that water deficit stress reduced plant height, internode length, total leaf area and weight, vegetative weight, number of squares, reproductive growth, as well as number and retention of bolls. On the other hand, drought increased specific leaf weight, chlorophyll content, and harvest index. The 1-MCP treatments had little or no positive effects on plant mapping, dry matter partitioning and chlorophyll content. The application of 1-MCP decreased the number of vegetative nodes, and increased the number of squares and reproductive nodes by 9% when plants were well-watered and by 17%, when under stress. The 1-MCP treatment showed a potential to improve lint yield in cotton, as it increased reproductive nodes per plant basis, mainly for cotton under water stress during its reproductive phase. However, this greater number of reproductive nodes did not lead to a better harvest index.

The objective of the field evaluations was determine the impact of 1-MCP on growth and yield components of cotton plants treated with ethephon as a source of abiotic stress under field conditions. Both rates of 1-MCP applied without surfactant accelerated crop maturity. Treatments containing only 1-MCP had significantly lower lint yields than the ethephon alone. However, it was revealed at harvest that both rates of 1-MCP showed potential to increase lint yield due to improvements in the upper canopy, but this potential was not translated into lint yield.

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